Clinical Isolates of *Aspergillus* Species Remain Fully Susceptible to Voriconazole in the Post-Voriconazole Era[▽]

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We studied the activity of voriconazole against 400 clinical strains of *Aspergillus* from the pre-voriconazole (1999 to 2002) and post-voriconazole (2003 to 2007) periods. Although the mean MICs of strains from the post-voriconazole period were slightly higher (0.39 versus 0.57 $\mu\text{g/ml}$; $P < 0.001$), all strains were susceptible to voriconazole and presented an MIC of $\leq 2 \mu\text{g/ml}$.

Based on both in vitro and clinical data, voriconazole has become the drug of choice for the treatment of invasive aspergillosis (1, 8, 10, 12).

The results of different in vitro studies showed that the vast majority of *Aspergillus* clinical strains are fully susceptible to the new triazoles, including voriconazole (1, 2, 8, 9, 11–13). However, the antifungal activity of voriconazole may have changed since it began to be used in the clinical setting.

We analyzed the in vitro antifungal activity of voriconazole against 400 clinical *Aspergillus* strains collected before and after its introduction in our institution (November 2002). We also examined the role of previous treatment with itraconazole and/or voriconazole in the appearance of strains of *Aspergillus* with diminished antifungal susceptibility to voriconazole.

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Organisms, source of samples, and period of study. The strains were from 281 patients, of whom 51 (18.1%) had proven or probable invasive aspergillosis according to the European Organization for Research and Treatment of Cancer

(EORTC) criteria. The species distribution of the strains analyzed was as follows: *Aspergillus fumigatus* ($n = 374$), *Aspergillus terreus* ($n = 20$), *Aspergillus niger* ($n = 3$), and *Aspergillus flavus* ($n = 3$). As for the source of the strains, 308 were from respiratory samples and 98 were from patients with invasive aspergillosis.

The isolates were grouped by period: those isolated during the period before the introduction of voriconazole (pre-voriconazole, 1999 to 2002) and those isolated after its introduction (post-voriconazole, 2003 to 2007). Both periods were comparable in terms of number of patients (143 versus 138), number of cases of proven/probable invasive aspergillosis (27 versus 24), and number of isolates (197 versus 203). Some patients had never received voriconazole or itraconazole, some had received it recently, and some were even taking it when the strains were isolated.

Analysis of the antifungal susceptibility of the strains. The antifungal activity of voriconazole (Pfizer Pharmaceutical Group, New York, NY) was determined by using the CLSI (formerly NCCLS) M38-A standard (4). All trays used in the assay were prepared at the same time, and all strains from both periods were tested using the same batch of trays.

TABLE 1. In vitro activity of voriconazole against clinical isolates of *Aspergillus* species^a

Period	Activity of voriconazole ^b ($\mu\text{g/ml}$) against:									
	All strains per period					Strains per patient group ^c				
	No. of strains	MIC ₉₀	MIC ₅₀	GM of MIC	Range	No. of patients	MIC ₉₀	MIC ₅₀	GM of MIC	Range
Pre-voriconazole	197	0.5	0.25	0.39	0.125–1	143	0.5	0.25	0.40	0.125–1
Post-voriconazole	203	1	0.5	0.57	0.125–2	138	1	0.5	0.61	0.125–2
Overall	400	1	0.5	0.48	0.125–2	281	1	0.5	0.51	0.125–2

^a Four hundred isolates were tested overall and for each study period using the CLSI M-38A procedure.

^b MIC endpoint for voriconazole and *Aspergillus* spp. was defined as the lowest concentration that produced complete inhibition of growth after 48 h of incubation. GM, geometric mean.

^c In patients with multiple isolates, only the highest MIC was chosen for analysis.

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FIG. 1. Distribution of the MICs of the strains during the pre-voriconazole and post-voriconazole periods. In those patients with multiple isolates, only the strain with the highest MIC was chosen.

Aspergillus resistance for voriconazole was classified as an MIC of ≥ 4 µg/ml.

Quality control was ensured by testing *A. flavus* ATCC 204304 and *A. fumigatus* ATCC 204305.

The antifungal susceptibilities of the strains, overall and per patient group, are shown in Table 1. In patients with multiple isolates, only the strain with the highest MIC was included. No voriconazole-resistant *Aspergillus* strains were detected (all isolates had MICs of ≤ 2 µg/ml). However, the mean MICs for strains from both periods were compared and were found to be significantly different (0.39 µg/ml versus 0.57 µg/ml; *t* test with a *P* value of <0.001): the MIC₉₀ and MIC₅₀ increased by a onefold dilution in the post-voriconazole period. We observed a creep toward higher MICs of voriconazole in the post-voriconazole period. During the pre-voriconazole period (only *A. fumigatus* strains were included), 92.9% of strains were inhibited at ≤ 0.5 µg/ml; in contrast, during the post-voriconazole period, only 78.5% were inhibited at ≤ 0.5 µg/ml (Fig. 1).

A. fumigatus strains proved to be more susceptible to voriconazole than other species of *Aspergillus* (0.46 µg/ml versus 0.76 µg/ml; *t* test with a *P* value of <0.001). However, we did not find differences in antifungal susceptibility for the source of the isolates (respiratory versus other sources, 0.49 µg/ml versus 0.46 µg/ml; *t* test with a *P* value of 0.289), the origin of the strains (infected versus noninfected patient, 0.47 µg/ml versus 0.49 µg/ml; *t* test with a *P* value of 0.630), or previous exposure to itraconazole or voriconazole (previous exposure versus no previous exposure, 0.46 µg/ml versus 0.48 µg/ml; *t* test with a *P* value of 0.733).

In order to determine whether the differences in susceptibility found between both periods could be explained by the presence of non-*A. fumigatus* strains only in the post-voriconazole period, we reanalyzed antifungal susceptibility to voriconazole including only strains of *A. fumigatus*. However, differences were still found between both periods (0.39 µg/ml versus 0.54 µg/ml; *t* test with a *P* value of <0.001).

Influence of antifungal susceptibility on outcome. Outcome was available for 47 of the 51 patients with proven or probable invasive aspergillosis: 26 patients received itraconazole or voriconazole (alone or in combination with other drugs), and 17 (36%) had a favorable outcome. We did not find a correlation between the presence of isolates with an MIC for voriconazole of ≥ 1 µg/ml and poor outcome (chi-square test with a *P* value

of 0.704). However, comparisons of outcomes associated with MICs did not account for other, multiple, competing host risks.

Our study showed that the strains of *Aspergillus* isolated from clinical samples in our institution from 1999 to the present remain fully susceptible to voriconazole. However, we detected a slight creep toward higher MICs for voriconazole in the strains isolated since it was introduced in our hospital in 2002. Although all isolates were tested with the same batch of voriconazole trays, we cannot rule out the possibility that these differences in susceptibility were due to variations within the assays.

In The Netherlands, Verweij et al. reported an increase in the isolation of *A. fumigatus* strains from patients with invasive aspergillosis showing resistance to several triazoles (14). The authors compared the prevalence of multiresistant strains during the periods of 1945 to 1998 and 1999 to 2002 (15). No multiresistant strains were found in the first period, but nine patients harbored them in the second period.

Although we did not find voriconazole-resistant strains, our analysis did reveal a trend toward the isolation of *Aspergillus* strains with slightly reduced susceptibility to voriconazole. We did not find differences between strains depending on whether or not the patients had previous exposure to anti-*Aspergillus* azoles. The role of the azoles in the development of resistance has not yet been clarified. Although some authors have reported a role of azoles in the development of resistance in *Aspergillus* (3, 5, 11, 15), other authors found itraconazole-resistant strains before exposure to the drug (7) or did not find resistant strains even in patients receiving azoles (6).

In conclusion, our study showed that all clinical strains of *Aspergillus* spp. isolated in our institution from 1999 to 2007 remain fully susceptible to voriconazole, with MICs of ≤ 2 µg/ml. Future studies are warranted to determine the current status of the susceptibility of clinical strains of *Aspergillus* to voriconazole in other institutions and countries.

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REFERENCES

1. Araujo, R., C. Pina-Vaz, and A. G. Rodrigues. 2007. Susceptibility of environmental versus clinical strains of pathogenic *Aspergillus*. *Int. J. Antimicrob. Agents* **29**:108–111.
2. Balajee, S. A., M. Weaver, A. Imhof, J. Gribskov, and K. A. Marr. 2004. *Aspergillus fumigatus* variant with decreased susceptibility to multiple antifungals. *Antimicrob. Agents Chemother.* **48**:1197–1203.
3. Chryssanthou, E. 1997. In vitro susceptibility of respiratory isolates of *Aspergillus* species to itraconazole and amphotericin B: acquired resistance to itraconazole. *Scand. J. Infect. Dis.* **29**:509–512.
4. Clinical and Laboratory Standards Institute/NCCLS. 2002. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved Standard. CLSI document M38-A. NCCLS, Wayne, Pennsylvania.
5. Dannaoui, E., E. Borel, M. F. Monier, M. A. Piens, S. Picot, and F. Persat. 2001. Acquired itraconazole resistance in *Aspergillus fumigatus*. *J. Antimicrob. Chemother.* **47**:333–340.
6. Dannaoui, E., J. Meletiadis, A. M. Tortorano, F. Symoens, N. Nold, M. A. Viviani, M. A. Piens, B. Lebeau, P. E. Verweij, and R. Grillot. 2004. Susceptibility testing of sequential isolates of *Aspergillus fumigatus* recovered from treated patients. *J. Med. Microbiol.* **53**:129–134.
7. Dannaoui, E., F. Persat, M. F. Monier, E. Borel, M. A. Piens, and S. Picot. 1999. In-vitro susceptibility of *Aspergillus* spp. isolates to amphotericin B and itraconazole. *J. Antimicrob. Chemother.* **44**:553–555.
8. Guinea, J., T. Peláez, L. Alcalá, M. J. Ruiz-Serrano, and E. Bouza. 2005. Antifungal susceptibility of 596 *Aspergillus fumigatus* strains isolated from outdoor air, hospital air, and clinical samples: analysis by site of isolation. *Antimicrob. Agents Chemother.* **49**:3495–3497.
9. Guinea, J., T. Peláez, S. Recio, M. Torres-Narbona, and E. Bouza. 2008. In vitro antifungal activities of isavuconazole (BAL4815), voriconazole, and fluconazole against 1,007 isolates of *Zygomycetes*, *Candida*, *Aspergillus*, *Fusarium*, and *Scedosporium* species. *Antimicrob. Agents Chemother.* **52**:1396–1400.
10. Herbrecht, R., D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J. W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlam, P. F. Troke, and B. de Pauw. 2002. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N. Engl. J. Med.* **347**:408–415.
11. Howard, S. J., I. Webster, C. B. Moore, R. E. Gardiner, S. Park, D. S. Perlin, and D. W. Denning. 2006. Multi-azole resistance in *Aspergillus fumigatus*. *Int. J. Antimicrob. Agents* **28**:450–453.
12. Manavathu, E. K., J. L. Cutright, D. Loebenberg, and P. H. Chandrasekar. 2000. A comparative study of the in vitro susceptibilities of clinical and laboratory-selected resistant isolates of *Aspergillus* spp. to amphotericin B, itraconazole, voriconazole and posaconazole (SCH 56592). *J. Antimicrob. Chemother.* **46**:229–234.
13. Mellado, E., G. García-Effrón, L. Alcázar-Fuoli, W. J. G. Melchers, P. E. Verweij, M. Cuenca-Estrella, and J. L. Rodríguez-Tudela. 2007. A new *Aspergillus fumigatus* resistance mechanism conferring in vitro cross-resistance to azole antifungals involves a combination of *cyp51A* alterations. *Antimicrob. Agents Chemother.* **51**:1897–1904.
14. Verweij, P. E., E. Mellado, and W. J. Melchers. 2007. Multiple-triazole-resistant aspergillosis. *N. Engl. J. Med.* **356**:1481–1483.
15. Verweij, P. E., D. T. Te Dorsthorst, A. J. Rijs, H. G. De Vries-Hospers, and J. F. Meis. 2002. Nationwide survey of in vitro activities of itraconazole and voriconazole against clinical *Aspergillus fumigatus* isolates cultured between 1945 and 1998. *J. Clin. Microbiol.* **40**:2648–2650.