

# In Vitro Susceptibility of *Aspergillus fumigatus* to Isavuconazole: Correlation with Itraconazole, Voriconazole, and Posaconazole

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**Triazoles are first-line agents for treating aspergillosis. The prevalence of azole resistance in *Aspergillus fumigatus* is increasing, and cross-resistance is a growing concern. In this study, the susceptibilities of 40 *A. fumigatus* clinical isolates were tested by using the CLSI method with amphotericin B, itraconazole, voriconazole, posaconazole, and the new triazole isavuconazole. Isavuconazole MICs were higher in strains with reduced susceptibilities to other triazoles, mirroring changes in voriconazole susceptibility. Isavuconazole MICs differed depending on the Cyp51A substitution.**

Triazoles are first-line antifungal agents for the treatment of invasive, chronic, and allergic diseases caused by *Aspergillus* spp. (1). There have been increasing reports of azole resistance in *Aspergillus fumigatus* (2–4). The clinical outcomes of infections caused by strains with reduced susceptibility to triazoles are frequently suboptimal (3, 5–8). Furthermore, the extent of a correlation (if any) between the *in vitro* susceptibilities of *Aspergillus* spp. to licensed azole compounds and newer agents, such as isavuconazole, is undefined (3, 7, 9, 10). The aim of this study was to determine the *in vitro* susceptibility to isavuconazole of a collection of 40 *A. fumigatus* isolates among which the majority had reduced susceptibility to itraconazole, voriconazole, and posaconazole.

All strains were clinical isolates obtained from the Mycology Reference Centre Manchester and were primarily isolated in the United Kingdom (but also included some strains from Canada, Denmark, United States, and France) between 1989 and 2008. The vast majority of isolates were obtained from cases following azole exposure (patients had received 1 to 30 months of azole therapy), although treatment was unknown in some cases. The majority (i.e., 33/40) of isolates had previously been demonstrated to possess an elevated MIC to at least one triazole, and a putative molecular mechanism of resistance had been defined (3, 4). The remaining 7 isolates were well-characterized clinical strains that demonstrated triazole MICs within the wild-type range, and all had wild-type *cyp51A* sequences except strain AF250, which had an N248K mutation.

MICs were determined using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology (11). Quality control strains *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used. MICs were estimated in three independently conducted experiments, and a consensus MIC value was established (i.e., the mode or median value; 96% were within 4-fold variation). The following compounds were tested: amphotericin B (Sigma, Poole, United Kingdom), itraconazole (Sigma), voriconazole (Pfizer Ltd., Sandwich, United Kingdom), posaconazole (Schering-Plough, New Jersey), and isavuconazole (Astellas Pharma Inc., Tokyo, Japan). The concentration range tested was 0.015 to 8 mg/liter for all compounds. For the purposes

of statistical analysis, MICs of >8 mg/liter were classified as 16 mg/liter.

The following epidemiological cutoff values (ECVs) within the CLSI methodology for *A. fumigatus* were used in the subsequent analysis:  $\leq 1$  mg/liter for itraconazole and voriconazole,  $\leq 0.25$  mg/liter for posaconazole, and  $\leq 4$  mg/liter for amphotericin (9, 12). There are no ECVs currently available for isavuconazole.

Thirty-two strains had triazole MICs above the ECV (9). Two strains had voriconazole MICs that straddled the ECV (1 to 2 mg/liter). One of these strains (F5211) had an MIC value that fell within the wild-type range in a previous study (3) but was above the ECV in this study, as the consensus MIC was slightly higher. The opposite was true for the other strain (F17764). An additional strain (F16867) had a reproducibly high itraconazole MIC (>8 mg/liter) in a previous study (3) but was consistently within the wild-type distribution (0.5 mg/liter) in this study. Potential reasons for this difference may be related to a change in methodology between the two studies from a modified EUCAST method (the modification was the use of a lower final inoculum concentration of  $0.5 \times 10^5$ , as opposed to  $1 \times 10^5$  to  $2.5 \times 10^5$  CFU/ml) versus the CLSI method, or loss of resistance during long-term storage at  $-80^\circ\text{C}$  (3). A molecular mechanism that could potentially account for elevated triazole MICs was not apparent for any of these isolates. The first two strains (F5211 and F17764) had a wild-type *cyp51A* sequence, and the latter (F16867) had a mutation known not to confer resistance (E427G), as it has been found in azole-susceptible strains. For the purposes of analysis, these strains were all classified in the wild-type group ( $n = 9$ ), leaving 31 non-wild-type strains with an MIC for at least one triazole agent that was above the ECV.

The isavuconazole MICs (geometric mean [range] of 0.93 mg/

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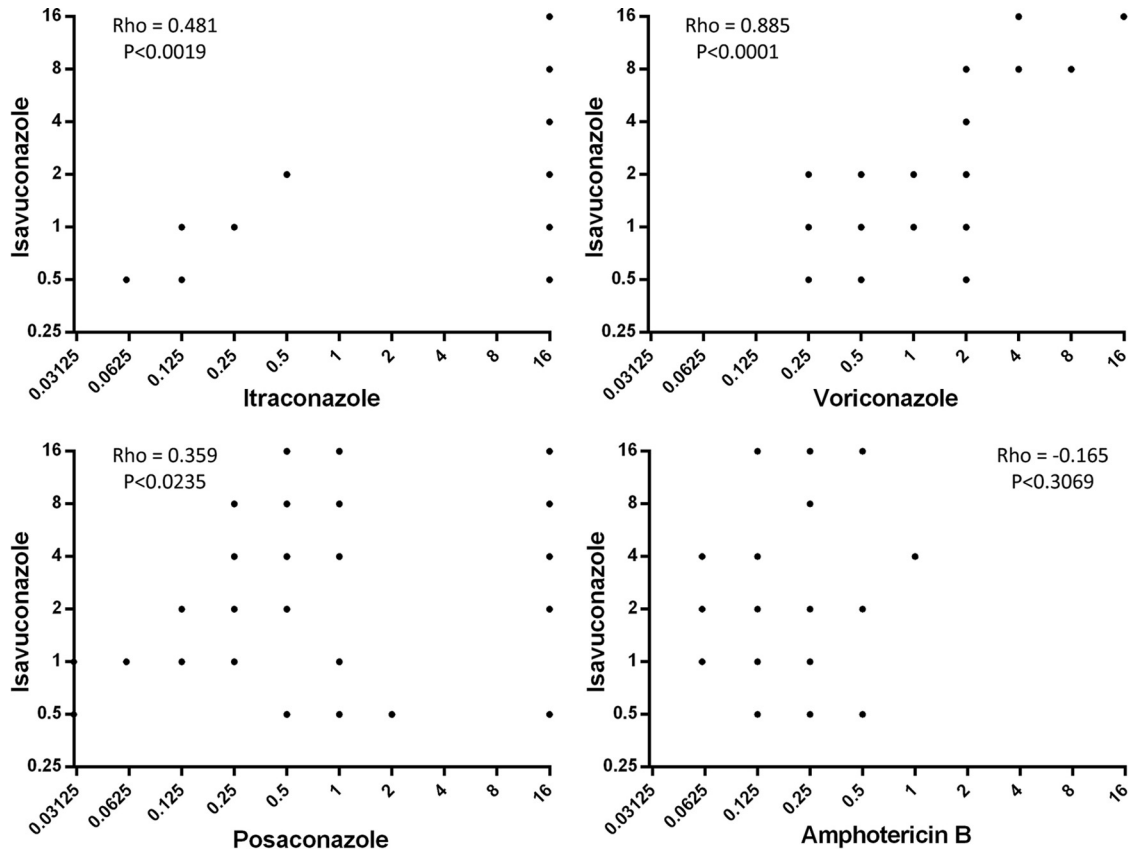


FIG 1 Relationship between isavuconazole *in vitro* susceptibility and susceptibility of the other antifungal drugs tested. The Spearman's rank correlation coefficient (rho) and statistical significance for each drug pair combination are shown within each graph.

liter [0.5 to 2 mg/liter]) of the wild-type strains were consistent with previous reports (13–15). Isolates with raised MICs to one or more triazole were more likely to have higher isavuconazole MICs. The geometric mean (range) of the non-wild-type strains was 3.66 mg/liter (0.5 to >8 mg/liter). There was a high degree of correlation between isavuconazole and voriconazole MICs ( $r = 0.885$  [Spearman's correlation coefficient];  $P < 0.001$ ) (Fig. 1). The extent of correlation between isavuconazole with itraconazole and posaconazole was less than that observed with voriconazole (Fig. 1), which corresponds with the drugs' structures (isavuconazole is similar in chemical structure to voriconazole, whereas posaconazole has a long side chain like itraconazole).

Amphotericin B susceptibility was within the wild-type range

for all isolates in this study ( $\leq 1$  mg/liter). The geometric mean for this drug did not significantly differ between the wild-type and non-wild-type groups (0.27 mg/liter versus 0.18 mg/liter, respectively), and the  $r$  value of  $-0.165$  was not statistically significant ( $P < 0.3069$ ), suggesting there was no correlation between amphotericin B and isavuconazole MICs.

Isolates with the alterations L98H, G138C, Y431C, G434C, and G448S showed elevated MICs to all triazoles, including isavuconazole (Table 1). Voriconazole and isavuconazole MICs were lower in isolates with amino acid substitutions at position G54, suggesting that alterations at this position only affect itraconazole and posaconazole susceptibility, as has been previously described (10). However, isavuconazole and voriconazole MICs were variable in

TABLE 1 MICs determined via the CLSI broth microdilution method

Susceptibility/Cyp51A alteration ( <i>n</i> )	MIC (mg/liter) geometric mean (range)				
	Amphotericin B	Itraconazole	Voriconazole	Posaconazole	Isavuconazole
Wild-type group (9)	0.27 (0.25–0.5)	0.15 (0.06–0.5)	0.58 (0.25–2)	0.06 (0.03–0.125)	0.93 (0.5–2)
Non-wild-type group (31)					
G54 (6)	0.18 (0.06–1)	16.00 (16)	2.00 (0.25–16)	1.02 (0.125–16)	3.66 (0.5–16)
L98 (3)	0.22 (0.06–0.5)	16.00 (16)	0.40 (0.25–2)	1.41 (0.5–16)	0.63 (0.5–2)
M220 (9)	0.25 (0.25)	16.00 (16)	5.04 (4–8)	0.63 (0.5–1)	10.08 (8–6)
G138/Y431/G434/G448 (5)	0.16 (0.06–1)	16.00 (16)	1.71 (0.5–4)	1.71 (0.25–16)	3.43 (1–8)
Others (8)	0.19 (0.125–0.5)	16.00 (16)	12.13 (4–16)	2.64 (0.5–16)	16.00 (16)
	0.16 (0.125–0.25)	16.00 (16)	1.83 (0.25–16)	0.30 (0.125–1)	4.00 (1–16)

isolates with M220 alterations. Additionally, specific amino acid substitutions can affect *in vitro* susceptibility (10). Therefore, isolates with a variety of different amino acid alterations were selected for this study, to investigate if there were any significant differences between substitutions. The only notable observation was that isolates with lysine substitutions at position 220 had higher posaconazole MICs than those with isoleucine, threonine, or valine alterations. However, the potential effects from other mechanisms of resistance contributing to this pattern of susceptibility cannot be discounted.

In summary, isavuconazole MICs were more likely to be higher in strains with reduced susceptibilities to other triazoles and had a high degree of correlation with voriconazole MICs. The extent to which diminished *in vitro* susceptibility has an impact on clinical outcome remains to be established. Therefore, additional *in vivo*, pharmacodynamic, and clinical data are required. The development of ECVs/breakpoints for isavuconazole against *Aspergillus* will aid in the interpretation of isavuconazole MICs.

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Isolates used in this study were provided by and are held in the clinical culture collection at The Mycology Reference Centre Manchester.

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