**Evaluation of isavuconazole MIC strips for susceptibility testing of *Aspergillus* and *Scedosporium* species**

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**Abstract**

Isavuconazole is a new triazole with an expanded-spectrum and potent activity against moulds and yeasts. It has been authorized for use in adults for the treatment of invasive aspergillosis and for mucormycosis. The only commercially available isavuconazole susceptibility test is the minimum inhibitory concentration (MIC) strip isavuconazole test. The objective of this study was to assess the *in vitro* activity of isavuconazole using gradient concentration MIC strips, compared with the EUCAST broth microdilution reference method. A total of 147 clinically relevant fungal isolates comprising 120 *Aspergillus* sp. and 27 *Scedosporium apiospermum* complex were tested for susceptibility to isavuconazole using the EUCAST broth microdilution method and by the MIC strip isavuconazole test. The percent essential agreement between the two methods was calculated within a 1-fold dilution. The geometric means for the MICs using the EUCAST reference methods and the strip test were respectively: 0.60 mg/l and 0.65 mg/l for *A. fumigatus*, 0.70 mg/l and 0.77 mg/l for *A. flavus*, 1.50 mg/l and 1.25 mg/l for *A. niger*, 0.41 mg/l and 0.38 mg/l for *A. terreus*, 1.22 mg/l and 1.08 mg/l for *S. apiospermum* complex. The isavuconazole MIC strips showed good agreement with the EUCAST reference method. Isavuconazole MIC strips could be useful for susceptibility testing of *Aspergillus* sp. and *S. apiospermum* complex.

**Key words:** antifungal susceptibility testing, isavuconazole, MIC strips test, EUCAST, *Aspergillus* and *Scedosporium*.

**Introduction**

Invasive fungal disease (IFD) is a major cause of mortality and morbidity in immunocompromised patients.1,2 Aspergillosis is the most common infection type caused by moulds in patients undergoing hematopoietic stem cell transplant or solid organ transplant and is associated with high mortality in these patient groups.1,2 Rare fungal species, such as *Scedosporium* spp., have limited treatment options and are associated with high mortality rates.3,4 In recent years the emergence of azole-resistant strains of *Aspergillus* spp. has been increasingly reported in patients under long-term antifungal treatment.5–9 However, even azole-resistant strains have been reported in naïve patients without any previous exposure toazole drugs, due to resistant strains acquired from the environment.10–12 Triazole antifungals drugs (i.e., itraconazole, voriconazole, posaconazole, and isavuconazole) are the major compounds currently involved in the treatment and prophylaxis of aspergillosis. In particular, isavuconazole is a triazole antifungal agent active against a broad range of clinically relevant fungi including *Aspergillus* spp.13–16 Based on the results from Phase 3 clinical trials, isavuconazonium sulfate has been approved by the US Food and Drug Administration for the treatment of adults with invasive aspergillosis (IA) and invasive mucormycosis.17,18 In addition, the European Commission has approved isavuconazole for the treatment of adults with IA and adults with mucormycosis for whom amphotericin B is not appropriate.19 A multicenter, randomized, double-blind, non-inferiority clinical trial compared the efficacy of isavuconazole versus voriconazole for the treatment of invasive fungal disease...
caused by Aspergillus spp. or other filamentous fungi. A total of 527 patients were randomized. The primary endpoint was all-cause mortality through day 42 in the intention-to-treat population. Isavuconazole was noninferior to voriconazole. Isavuconazole was also better tolerated by patients and showed a better safety profile. However, only three patients with Scedosporium infection were enrolled, thus precluding conclusions about efficacy for these infections. The management of these complex infections request the individualized care of patients with Scedosporium, including susceptibility testing of the isolate with clinical course. In vitro activity of isavuconazole has shown against some isolates of S. apiospermum, but because of the different activity of antifungal agents against S. apiospermum and especially Lomentospora prolificans, it is usually useful to ask for the susceptibility testing of isolates in patients with infections caused by these pathogens. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has set clinical breakpoints for isavuconazole and Aspergillus. In particular, for A. fumigatus the currently proposed clinical breakpoint is 1 mg/liter derived on epidemiological cutoff value (ECOFF) (2 mg/liter) and in vitro pharmacokinetic (PK)/pharmacodynamic (PD) studies. Also for A. terreus the currently clinical breakpoint is 1 mg/liter with an ECOFF of 1 mg/liter. Instead, interpretable clinical breakpoints for antifungal MICs and Scedosporium complex are not available.

The only commercially available isavuconazole susceptibility test is the MIC strip isavuconazole test (Liofilchem, Roseto degli Abruzzi, TE, Italy). In a study on evaluation of MIC strip isavuconazole test for susceptibility testing of Aspergillus fumigatus isolates was been highlighted a greater agreement with EUCAST using the full inhibition endpoint.

The objective of this study was to assess the in vitro activity of isavuconazole comparing gradient minimum inhibitory concentration (MIC) strips with the EUCAST broth microdilution reference method.

Methods

In total, we evaluated the MICs of 147 clinically relevant fungi such as Aspergillus fumigatus (n = 48), Aspergillus flavus (n = 40), Aspergillus niger (n = 14), Aspergillus terreus (n = 18) and Scedosporium apiospermum complex (n = 27) isolates from patients admitted to Intensive Care Unit, and Hematology, Pneumology, Pediatric Pneumology, and Medicine were included. The isolates were obtained from various respiratory tract specimens and in particular bronchoalveolar lavage, bronchial aspirate and sputum. Identification was done using macro- and micromorphology, supplemented by thermo-tolerance (incubation at 37°C and 43°C) and β-tubulin sequencing for cryptic Aspergillus spp. The use of the term “complex” is acknowledged for Aspergillus spp. other than A. fumigatus, in the absence of detailed molecular identification, although for simplicity, it is not used throughout this article. All isolates were tested for susceptibility to isavuconazole using the EUCAST broth microdilution method (E. Def 9.3) and by MIC strip isavuconazole test (Liofilchem, Roseto degli Abruzzi, TE, Italy). For EUCAST susceptibility testing, stock solution (5000 mg/l in dimethyl sulphoxide; Sigma-Aldrich, Brøndby, Denmark) of isavuconazole (Basilea Pharmaceutica Ltd., Basel, Switzerland) was prepared. Final drug concentration ranges for isavuconazole was 0.125–16 mg/l. MICs were determined visually applying a no growth endpoint after 48 hours of incubation (Aspergillus), as recommended. EUCAST control strains Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were routinely included and read after 24 hours. For interpretation of susceptibility, the following EUCAST clinical breakpoints were used for A. fumigatus and A. terreus: isavuconazole MIC ≤ 1 mg/l (susceptible) and > 1 mg/l (resistant). For the strip test cultures were prepared in a MacFarland 0.5 conidia suspension and grown on RPMI 1640 2% glucose agar gel (SSI Diagnostica, Hillerød, Denmark). MICs from the strips were read at 80% growth inhibition after 48 hours incubation at 35°C. For the analysis of essential agreement, values from the MIC strips were rounded up to the next corresponding dilution for the broth microdilution. The percent of essential agreement between the two methods was calculated within ± one fold dilution. The categorical agreement between the methods was calculated as the percentage of isolates classified equally by both methods. Very major errors (VMEs) were defined as isolate categorization as resistant (R) by EUCAST but susceptible (S) by the MIC strip test, and major errors (MEs) were defined as isolate categorization as S by the EUCAST method but R by the MIC strip test. Data were analyzed using the MedCalc Statistical Software version 17.9.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017).

Results

A total numbers and MIC distributions for Aspergillus spp. and Scedosporium apiospermum complex tested in the study is provided in Table 1. The MICs for Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were in the recommended ranges. Applying the clinical breakpoints for the A. fumigatus isolates that underwent EUCAST susceptibility testing and MIC test strip, the susceptibility profiles were respectively as follows: 8.3% and 6.2% (resistant; R), 91.7% and 93.7% (susceptible; S). For the A. terreus isolates the susceptibility profiles were: 5.5% resistant and 94.4% susceptibility for both methods. The geometric means for the MICs using the EUCAST reference methods and the MIC strip test were respectively: 0.60 mg/l and 0.65 mg/l for A. fumigatus, 0.70 mg/l and 0.77 mg/l for A. flavus, 1.50 mg/l and 1.25 mg/l for A. niger, 0.41 mg/l and 0.38 mg/l for A. terreus, 1.22 mg/l and 1.08 mg/l for S. apiospermum complex. The percentage of MICs above the ECOFFs for the two methods (EUCAST/MIC strip test) were as follows: A. fumigatus (0/0)
Table 1. Isavuconazole susceptibility of Aspergillus spp. and Scedosporium apiospermum complex determined by EUCAST and MIC strip test.

<table>
<thead>
<tr>
<th>Species</th>
<th>N (%)</th>
<th>EUCAST (E. Def. 9.3)</th>
<th>MIC strip test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC range</td>
<td>MIC 50</td>
<td>MIC 90</td>
</tr>
<tr>
<td>A. fumigatus sensu stricto</td>
<td>48 (32.6%)</td>
<td>0.125 - 2</td>
<td>1</td>
</tr>
<tr>
<td>A. flavus species complex</td>
<td>40 (27.4%)</td>
<td>0.125 - 2</td>
<td>1</td>
</tr>
<tr>
<td>A. niger species complex</td>
<td>14 (9.5%)</td>
<td>0.5 - 8</td>
<td>1.5</td>
</tr>
<tr>
<td>A. terreus species complex</td>
<td>18 (12.2%)</td>
<td>0.125 - 2</td>
<td>0.5</td>
</tr>
<tr>
<td>S. apiospermum complex</td>
<td>27 (18.4%)</td>
<td>0.5 - 8</td>
<td>1</td>
</tr>
<tr>
<td>C. krusei ATCC 6258</td>
<td>...</td>
<td>0.015 - 0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>C. parapsilosis ATCC 22019</td>
<td>...</td>
<td>0.008 - 0.03</td>
<td>0.015</td>
</tr>
</tbody>
</table>

ECOFF, epidemiological cutoff value; MIC50, MIC value inhibiting the growth of >50% of isolates; MIC90, MIC value inhibiting the growth of >90% of isolates; NA, not available.
Discussion

Isavuconazole is a new extended-spectrum triazole with activity against yeasts, moulds, and dimorphic fungi. The safety profile of this agent and its excellent pharmacokinetic characteristics make isavuconazole an attractive option for treatment of invasive fungal infections. Similar to other azoles, isavuconazole inhibits cytochrome P450 (CYP)–dependent 14α-lanosterol demethylation, which is essential for fungal cell membrane ergosterol synthesis. This blockade produces methylated sterols in the fungal membrane, altering its function and allowing the accumulation of ergosterol toxic precursors in the cytoplasm, which leads to cell death. The side arm of the active isavuconazole molecule allows for greater affinity of isavuconazole for the binding pocket in the fungal CYP51 protein increasing the antifungal spectrum and conferring activity against some isolates resistant to other triazoles. The in vitro activity of isavuconazole has been demonstrated against most Aspergillus spp. including A. fumigatus, A. flavus, A. terreus, and A. niger. In recent years, the emergence of azole resistant Aspergillus spp. has become a threat to patients with the majority of clinical cases to date reported from western Europe. In these resistant isolates, mutations within the CYP51A gene (which encodes the enzyme targeted by azoles) or within the gene promoter, have been identified that reduce drug affinity or increase target quantity conferring azole resistance. In Aspergillus isolates, cross-resistance to triazoles is quite variable and related to the type of mutations. Therefore it would be useful the detection of in vitro susceptibility test to the triazoles and the detection of the corresponding mutations within the CYP51A gene or within the gene promoter.

Our study assessed the in vitro activity of isavuconazole using gradient concentration MIC strips, compared with the EUCAST broth microdilution reference method. According to EUCAST breakpoints 8.3% and 5.5% of all A. fumigatus and A. terreus isolates were isavuconazole resistant. The categorical agreement for A. fumigatus and A. terreus was >97% when interpreting the MICS according to EUCAST breakpoints, and notably, in one isolate of A. fumigatus a VME was found.

In this strain it could be useful to study the resistance mechanism considering that the isolates with a wild-type CYP51A target gene either may be harboring other resistance mechanisms or may be isolates that are truly susceptible but classified as R by the EUCAST reference method because of the conservative EUCAST susceptibility breakpoint. In all of the A. flavus strains tested with both methods they were observed MIC values below the epidemiological cutoff (2 mg/l). In one isolate of A. niger a discrepancy between the MIC strip test and EUCAST method was found. In particular, in this isolate the MIC value obtained by MIC strip test were above the epidemiological cutoff than those obtained by EUCAST method. Finally, isavuconazole MICs obtained with MIC test strip and EUCAST method against S. apiospermum complex were comparable. Moreover, for Scedosporium apiospermum complex could be useful distinguish Scedosporium species (S. apiospermum, S. boydii, S. minutissorum, S. aurantium, S. debooei, etc.) by molecular biology considering that these species have variable susceptibility to antifungal.

In conclusion the isavuconazole MIC strips could be useful for susceptibility testing of Aspergillus spp. and S. apiospermum complex, also evaluating the strip full inhibition endpoint since the essential and categorical agreements appear best with EUCAST method. Further studies are needed to increase the isavuconazole in vitro data and to define clinical breakpoints for other Aspergillus species. Furthermore, the appropriate use of antifungal drugs could reduce the possible development of resistance mechanisms showed in some moulds isolated in critically ill patients.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References


