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ORIGINAL ARTICLE

Antifungal Activity of Efinaconazole Compared to Fluconazole, Itraconazole, and Terbinafine against Terbinafine- and Itraconazole-Resistant and -Susceptible Clinical Isolates of Dermatophytes, Candida, and Mold

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Background: Recently, an increasing number of resistant-to-terbinafinedermatophytosis cases have been reported. Thus, identifying an alternative antifungal agent that possesses a broad-spectrum activity, including against resistant strains, is needed.

Methods: In this study, we compared the antifungal activity of efinaconazole to fluconazole, itraconazole, and terbinafined against clinical isolates of dermatophyte, Candida, and molds using in vitro assays. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of each antifungal was quantified and compared. Both susceptible and resistant clinical isolates of Trichophyton mentagrophytes (n=16), T. rubrum (n=43), T. tonsurans (n=18), T. violaceum (n=4), Candida albicans (n=55), C. auris (n=30), Fusarium sp., Scedosporium sp., and Scopulariopsis sp. (n=15 for each) were tested.

Results: Our data shows that efinaconazole was the most active antifungal, compared to the other agents tested, against dermatophytes with MIC50 and MIC90 (Concentration that inhibited 50% and 90% of strains tested, respectively) values of 0.002 and 0.03 μg/ml, respectively.
Fluconazole, itraconazole and terbinafine showed \( \text{MIC}_{50} \) and \( \text{MIC}_{90} \) values of 1 and 8 \( \mu \text{g/ml} \), 0.03 and 0.25 \( \mu \text{g/ml} \), and 0.031 and 16 \( \mu \text{g/ml} \), respectively. Against *Candida* isolates, efinaconazole \( \text{MIC}_{50} \) and \( \text{MIC}_{90} \) values were 0.016 and 0.25 \( \mu \text{g/ml} \), respectively, whereas fluconazole, itraconazole and terbinafine had \( \text{MIC}_{50} \) and the \( \text{MIC}_{90} \) values of 1 and 16 \( \mu \text{g/ml} \), 0.25 and 0.5 \( \mu \text{g/ml} \), and 2 and 8 \( \mu \text{g/ml} \), respectively. Against various mold species, efinaconazole MIC values ranged from 0.016 and 2 \( \mu \text{g/ml} \), compared to 0.5 to greater than 64 \( \mu \text{g/ml} \) for the comparators.

**Conclusions:** Efinaconazole showed superior potent activity against a broad panel of susceptible and resistant dermatophyte, *Candida*, and mold isolates.

Superficial fungal infections are common and may involve the upper layer of the skin and/or its appendages, such as fingernails and toenails. [1] These infections may offer distinct clinical presentations depending on the causative fungal species. [2] Dermatophytes mainly infect keratinized tissue and are the main cause of superficial fungal infections known as dermatophytosis. [3] *Trichophyton rubrum* is the most common species causing dermatophytic infections. [4] Superficial fungal infections may also result from non-dermatophytic fungi including yeast (e.g., *Candida albicans* and non-*Candida* species such as *C. glabrata*), and less commonly mold (e.g., *Scopulariopsis brevicaulis* *Fusarium*, and *Aspergillus* species). [5-7]
Several factors play a significant role in treatment selection for superficial fungal infections. For example, localized infections are more likely to be treated with topical antifungal preparations such as azoles and ciclopirox. While widespread and deep infections (i.e., infections extending into follicles or the dermis) are less likely to respond to topical treatments, and often require oral drugs such as terbinafine, itraconazole, fluconazole, or griseofulvin. The selection of an antifungal agent is dependent on the type and susceptibility pattern of the organism(s) cultured from the infected site.

Increasing resistance to commonly prescribed antifungal agents has become a challenging issue that physicians often encounter. Such resistance has been observed in various fungal groups including yeast and molds prompting the Center for Disease Control and Prevention to issue a warning regarding the alarming trend of resistance to commonly available antifungals. This concern increased following the isolation of Candida auris, an emerging multidrug resistant Candida species that demonstrated multidrug as well as pan-resistance to different classes of marketed antifungal drugs.

In addition to C. auris, there has been a reported increase in resistance to terbinafine and azoles among dermatophytes. For example, in India, dermatophytosis outbreaks resulting from resistant strains have been reported in numerous areas and has been categorized as an epidemic. Although uncommon, resistance has also been increasing in
frequency in the U.S., and demonstrates resistance to oral terbinafine or second-line systemic therapies (oral fluconazole or itraconazole). [30]

Efinaconazole is a triazole that inhibits fungal lanosterol 14α-demethylase, thereby decreasing biosynthesis of ergosterol. [31] Efinaconazole was first approved by the U.S. Food and Drug Administration (FDA) in 2014 as a topical treatment for onychomycosis caused by T. rubrum and T. mentagrophytes. [32] It has been previously reported that different azoles exhibit variable rates of lanosterol 14α-demethylase inhibition as shown by lower ergosterol content in Candida cells in the presence of voriconazole compared to fluconazole. [33] We hypothesized that efinaconazole may be effective against isolates with known resistance against itraconazole and/or terbinafine.

In this study, we compared the antifungal activity of efinaconazole to fluconazole, itraconazole, and terbinafine using terbinafine- and itraconazole-resistant and susceptible dermatophytes, yeast, and mold clinical isolates.

Materials and Methods

Fungal Species Tested:

The following susceptible and resistant fungal strains were used in this study:

Dermatophyte species tested were Trichophyton mentagrophytes (n=16), Trichophyton rubrum (n=43), Trichophyton tonsurans (n=18), and Trichophyton violaceum (n=4). Yeast: Candida
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*albicans* (n=55), and *Candida auris* (n=30), and three non-dermatophytes mold species: *Fusarium* sp. (n=15), *Scedosporium* spp. (n=15), and *Scopulariopsis* spp. (n=15)

**Antifungal Agents:**

Four antifungal agents were evaluated: efinaconazole, fluconazole, itraconazole, and terbinafaine. Efinaconazole was provided by Bausch Health pharmaceutical company.

Fluconazole, itraconazole, and terbinafaine were purchased from Sigma–Aldrich (St Louis, MO, USA).

**Minimum Inhibitory Concentration (MIC):**

MIC testing was performed according to the Clinical and Laboratory Standard Institute (CLSI) microdilution methods for yeasts (Document M27Ed4E), and for dermatophytes and non-dermatophyte mold (Document M38Ed3). [34,35] Antifungal agents were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO) and two-fold serial dilutions were prepared in RPMI 1640 (Hardy Diagnostics, Santa Maria, CA) and added to wells of a 96-well microdilution plate. The inoculate for dermatophytes, non-dermatophyte molds and yeasts were prepared in RPMI 1640 to concentrations of 1-3 × 10^3, 0.4- 5 × 10^4 and 0.5-2.5 × 10^3 colony forming units (CFUs)/mL, respectively and added to the drug dilutions in a 96 well plate. MIC microdilution plates for dermatophytes, non-dermatophyte molds, and yeasts were incubated at 35°C for 24, 48 and 96 hours, respectively. The MIC inhibition endpoint for dermatophytes was the lowest
concentration of antifungal exhibiting an 80% reduction in growth compared to the untreated growth control. While MIC inhibition endpoints for non-dermatophyte molds and yeasts were recorded at 50% inhibition compared to growth control, except itraconazole versus molds where the endpoint was 100% inhibition. The quality control isolates C. parapsilosis (ATCC 22019) and C. krusei (ATCC 6258) were tested in parallel for each test series.

Minimum Fungicidal Concentration (MFC):

MFC determinations were performed according to the method previously described by Ghannoum and Isham. [36] The total contents of each well showing no visible growth during the MIC assay were sub-cultured onto a potato dextrose agar plates. To avoid antifungal carryover, the aliquots were allowed to soak into the agar and the agar surface was streaked for isolation once dry, thus removing the cells from the drug source. Inoculated plates were then incubated at 35 °C for 24 hours for yeast, 48-72 hours for mold, and 96 hours for dermatophyte. The number of CFUs were then determined. Fungicidal activity was defined as a \( \geq 99.9\% \) reduction in the number of CFUs/ml from the starting inoculum count. While fungistatic activity was defined as \(< 99.9\% \) reduction.
Results

1. **Activity of Antifungals Against Dermatophytes:**

   Against combined dermatophyte isolates (n=81), efinaconazole was the most potent antifungal with MIC\(_{50}\) and MIC\(_{90}\) values of 0.002 and 0.03 μg/mL, respectively. Fluconazole showed MIC\(_{50}\) and MIC\(_{90}\) values of 1 and 8 μg/mL, respectively, and the MIC\(_{50}\) and MIC\(_{90}\) values for itraconazole and terbinafine were 0.03 and 0.25 and 0.031 and 16 μg/mL, respectively (Table 1- upper panel).

Table 1. Minimum Inhibitory Concentration of efinaconazole and comparators against Dermatophyte isolates (μg/mL).

<table>
<thead>
<tr>
<th></th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Terbinafine</th>
<th>Efinaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichophyton sp.</strong> (n=81)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>≤0.125 - &gt; 64</td>
<td>≤ 0.016 - 1</td>
<td>≤ 0.001 - &gt; 64</td>
<td>≤ 0.001 - 0.25</td>
</tr>
<tr>
<td>MIC(_{50})</td>
<td>1</td>
<td>0.03</td>
<td>0.03</td>
<td>0.002</td>
</tr>
<tr>
<td>MIC(_{90})</td>
<td>8</td>
<td>0.25</td>
<td>16</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>T. rubrum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>≤ 0.125 - &gt; 64</td>
<td>≤ 0.016 - 0.5</td>
<td>0.002 - &gt; 64</td>
<td>≤ 0.001 - 0.063</td>
</tr>
<tr>
<td>MIC(_{50})</td>
<td>0.5</td>
<td>0.03</td>
<td>0.5</td>
<td>0.002</td>
</tr>
<tr>
<td>MIC(_{90})</td>
<td>2</td>
<td>0.06</td>
<td>16</td>
<td>0.031</td>
</tr>
<tr>
<td>Species</td>
<td>Range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------</td>
<td>------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>T. tonsurans</td>
<td>≤ 0.125 - 8</td>
<td>0.25</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>(n=18)</td>
<td>≤ 0.016 - 0.25</td>
<td>0.03</td>
<td>0.06</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>≤ 0.001 - 32</td>
<td>0.001 - 0.063</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>≤ 0.125 - 32</td>
<td>0.063</td>
<td>0.031</td>
<td>0.016</td>
</tr>
<tr>
<td>(n=16)</td>
<td>≤ 0.016 - 0.25</td>
<td>0.004 - 16</td>
<td>0.001 - 0.25</td>
<td></td>
</tr>
<tr>
<td>T. violaceum</td>
<td>1 - 8</td>
<td>0.125 -1</td>
<td>0.016 -0.063</td>
<td>0.004 -0.25</td>
</tr>
<tr>
<td>(n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the present study, 27 dermatophyte isolates (T. rubrum =22, T. mentagrophytes =4 and T. tonsurans =1) showed elevated MIC values against terbinafine with a range of 0.5 - > 64 μg/mL, MIC<sub>50</sub> value of 4 μg/mL and MIC<sub>90</sub> value of 32 μg/mL. Against these isolates, efinaconazole showed more potent activity compared to the other tested drugs with an MIC range of ≤ 0.001 – 0.25 μg/mL, MIC<sub>50</sub> value of 0.002 μg/mL and MIC<sub>90</sub> value of 0.031 μg/mL. For
itraconazole and fluconazole, MIC\textsubscript{50} and MIC\textsubscript{90} values were 0.03 and 0.25, and 0.5 and 8 µg/mL, respectively (Table 2).

Table 2. Minimum Inhibitory Concentration of efinaconazole against terbinafine less susceptible dermatophyte isolates (n=27)

<table>
<thead>
<tr>
<th></th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Terbinafine</th>
<th>Efinaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>≤ 0.125 - 32</td>
<td>≤ 0.016 – 0.5</td>
<td>0.5 - &gt; 64</td>
<td>≤ 0.001 - 0.25</td>
</tr>
<tr>
<td>MIC\textsubscript{50}</td>
<td>0.5</td>
<td>0.03</td>
<td>4</td>
<td>0.002</td>
</tr>
<tr>
<td>MIC\textsubscript{90}</td>
<td>8</td>
<td>0.25</td>
<td>32</td>
<td>0.03</td>
</tr>
</tbody>
</table>

2. Activity of Antifungals Against Yeast:

The MICs of the test compounds against Candida species are summarized in Table 3.

The MIC\textsubscript{50} and MIC\textsubscript{90} values were 0.016 and 0.25 µg/mL, respectively for efinaconazole. While MIC\textsubscript{50} and MIC\textsubscript{90} values for fluconazole were 1 and 16 µg/mL, for itraconazole were 0.25 and 0.5 µg/mL, and for terbinafine were 2 and 8 µg/mL, respectively. Interestingly, efinaconazole was the most effective agent against C. auris isolates tested in our study.

Table 3. Minimum Inhibitory Concentration of tested compounds against Candida isolates (µg/mL)

In the current study, four *C. albicans* isolates had markedly elevated MICs when tested against terbinafine (range of 16 - >64 μg/mL), whereas efinaconazole had higher activity against the same isolates with an MIC range of 0.008 – 2 μg/mL (Table 4). Furthermore, four isolates (*C. albicans* and *C. auris*) had an MIC range of 1 - > 64 μg/mL against itraconazole compared to a range of 0.5 - 32 μg/mL against efinaconazole. Finally, three *C. albicans* isolates that exhibited elevated MIC values against both terbinafine and itraconazole were more susceptible to efinaconazole (Table 4).
Table 4. Activity of efinaconazole against *Candida* strains with high MIC values against terbinafine and itraconazole (µg/mL).

<table>
<thead>
<tr>
<th>Organism</th>
<th>MRL number</th>
<th>#</th>
<th>Terbinafine</th>
<th>Itraconazole</th>
<th>Efinaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>42615</td>
<td>&gt; 64</td>
<td></td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>2996</td>
<td>&gt; 64</td>
<td></td>
<td>0.063</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>11031</td>
<td>&gt; 64</td>
<td></td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>11040</td>
<td>16</td>
<td></td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td><em>C. auris</em></td>
<td>13017</td>
<td>1</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>29037</td>
<td>&gt;64</td>
<td></td>
<td>32</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>27884</td>
<td>&gt;64</td>
<td></td>
<td>32</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>28975</td>
<td>&gt;64</td>
<td></td>
<td>32</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>33759</td>
<td>64</td>
<td>1</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>42528</td>
<td>&gt; 64</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>21547</td>
<td>&gt; 64</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

- Organisms with high MIC against terbinafine
- *Organisms with high MIC against itraconazole
- *Organisms with high MIC against terbinafine and itraconazole
3. Activity of Antifungals Against Molds:

Table 5 shows the antifungal activity against different types of molds. MIC$_{50}$ and MIC$_{90}$ values for efinaconazole against *Scedosporium*, *Fusarium* spp., and *Scopulariopsis* spp. were 0.063 and 0.125 μg/mL, 0.5 and 2 μg/mL, and 0.125 and 0.25 μg/mL, respectively. Furthermore, the MIC$_{50}$ and MIC$_{90}$ values for fluconazole against *Scedosporium*, *Fusarium* and *Scopulariopsis* ranged between 16 -> 64 and 64 -> 64 μg/mL, respectively.

Although, MIC$_{50}$ and MIC$_{90}$ values for terbinafine were elevated against *Scedosporium* (> 64 μg/mL for both) at the 72h incubation period, relatively lower values were seen against *Fusarium* and *Scopulariopsis* strains tested (2 and 16 μg/mL, respectively and 1 and 2 μg/mL, respectively) at the 48h incubation period. (Table 5)

Table 5. Minimum Inhibitory Concentration of test compounds against mold isolates (μg/mL)

<table>
<thead>
<tr>
<th></th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Terbinafine</th>
<th>Efinaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scedosporium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2-64</td>
<td>1 -&gt; 64</td>
<td>2 -&gt; 64</td>
<td>0.016 - 0.5</td>
</tr>
<tr>
<td>(n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC$_{50}$</td>
<td>16</td>
<td>&gt; 64</td>
<td>&gt; 64</td>
<td>0.063</td>
</tr>
<tr>
<td>MIC$_{90}$</td>
<td>64</td>
<td>&gt; 64</td>
<td>&gt; 64</td>
<td>0.125</td>
</tr>
<tr>
<td>Range</td>
<td>16 -&gt; 64</td>
<td>&gt; 64</td>
<td>0.5-16</td>
<td>0.125 -2</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th></th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Range</th>
<th>0.5-&lt;sup&gt;→&lt;/sup&gt;</th>
<th>0.063 - 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fusarium spp.</strong></td>
<td>&gt; 64</td>
<td>&gt; 64</td>
<td>2</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>(n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Scopulariopsis</strong></td>
<td>Range</td>
<td>&gt; 64</td>
<td>0.5-&lt;sup&gt;→&lt;/sup&gt;</td>
<td>0.063 - 0.5</td>
<td></td>
</tr>
<tr>
<td>(n=15)</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>&gt; 64</td>
<td>1</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>&gt; 8</td>
<td>2</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

Fungicidal Activity of Tested Antifungals:

Our data showed that in general, none of the tested compounds showed fungicidal activity against isolates from different species. However, efinaconazole demonstrated more fungicidal activity against *T. rubrum* isolates compared to other antifungal agents with an MFC range of 0.002 - > 0.5 µg/mL and MIC<sub>50</sub> value of 0.5 µg/mL.
Discussion

Recently, reports of terbinafine-resistant infections have been trending up across the globe, especially from patients in India. [39-42] In the present study, efinaconazole demonstrated the highest inhibitory activity against dermatophytes compared to other antifungal agents, including 27 isolates that had elevated MICs against terbinafine. The observed results support data reported by Hur et al. in which efinaconazole had a similar or higher activity compared to terbinafine. [43] Although efinaconazole and terbinafine showed the most potent effect against dermatophytes (T. rubrum and T. mentagrophyte) compared to the other agents, efinaconazole had a higher activity against 10 out of 63 T. rubrum and 8 out of 59 T. mentagrophyte isolates compared to terbinafine (MIC values of 0.0005- 0.125 vs ≥ 1 μg/mL). [43] Similarly, Rezaei-Matehkolaie et. al. also showed that efinaconazole demonstrated a comparable antifungal activity against T. rubrum (n=54) with an MIC range of 0.002–0.06 μg/mL compared to 0.004–0.06 μg/mL for terbinafine. [44]

In a detailed study of 1,387 T. rubrum and 106 T. mentagrophytes isolates obtained from onychomycosis patients in the United States, Canada, and Japan, the MICs for efinaconazole against T. rubrum and T. mentagrophytes ranged between ≤0.002–0.03 and ≤0.002 to 0.06 μg/mL, respectively compared to 0.004–0.06 and 0.004–0.5 μg/mL, respectively for terbinafine. [45] In addition, itraconazole inhibited the fungal growth at relatively higher
MICs, ranging between 0.015–0.125 μg/mL for \( T. \) \( \text{rubrum} \) and 0.03–0.25 μg/mL for \( T. \) \( \text{mentagrophytes} \). Interestingly, comparing the susceptibility profile of 10 isolates before treatment with efinaconazole versus after therapy (48 weeks) was associated with a minimal increase in the MIC values (the greatest change was from ≤0.002 to 0.008 μg/mL). This may suggest that development of antifungal resistance is less likely to occur with prolonged efinaconazole therapy. [45]

We also tested the antifungal activity of efinaconazole against other non-dermatophyte fungi implicated in superficial fungal infections (\( C. \) \( \text{andida} \) and molds). Efinaconazole was the most active compound against \( C. \) \( \text{andida} \) species compared to the other antifungals tested including isolates with elevated MIC values against itraconazole and terbinafine. A similar observation was reported by Jo Siu \( et \) \( al. \), who tested efinaconazole activity against 105 \( C. \) \( \text{femalbicans} \) isolates. Efinaconazole MICs ranged between <0.0005 and >0.25 μg/mL, with 50% of isolates being inhibited by 0.001 μg/mL after 24 hours incubation compared to 0.06 – >16 and ≤0.004 – >2 μg/mL for terbinafine and itraconazole, respectively. [45] Along the same line, the data provided by Hur \( et \) \( al. \) also showed that efinaconazole had a lower mean MIC value for efinaconazole compared to itraconazole, terbinafine, amorolfine, and ciclopirox. (0.001 vs. 0.015, 64.041, 73.640, and 3.254 μg/mL, respectively). [43]
In the present study, four *C. albicans* isolates demonstrated markedly elevated MIC values against terbinafine (range between 16 - >64 μg/mL), another four isolates had elevated MIC against itraconazole (MIC range of 1- > 64 μg/mL) [46], and three *C. albicans* isolates exhibited reduced susceptibility against both agents (MIC range of 64 - > 64 and 1 – 4 μg/mL, respectively). Efinaconazole, on the other hand, had lower MIC values against all the isolates that were more resistant to terbinafine, which is not surprising as terbinafine has been widely reported to exhibit sub-optimal activity against different *Candida* spp. [47] Additionally, against isolates resistant to itraconazole, efinaconazole had higher inhibitory activity. It is important to note that the MIC values of both agents (itraconazole versus efinaconazole) differed by only 1 to 3 micro dilutions. Given that cross-resistance between different azoles has been reported, the observed results are reasonable. [48,49]

The mechanism by which triazole members, such efinaconazole and itraconazole, may exhibit different efficacy against the same isolates is still being investigated. However, different theories have been proposed including that different triazoles inhibit lanosterol 14α-demethylase (the targeted enzyme) activity to variable degrees. [33] Others suggested that differences in resistance is largely the result of a decreased susceptibility of 14α-demethylase to the inhibitory effects of a given triazole (e.g., fluconazole). [50]
An interesting observation in our study is that efinaconazole exhibited high activity against the multidrug resistant species *C. auris* compared to the other tested agents. *C. auris* is reported to demonstrate resistance against a wide range of commercially available antifungal drugs. [18] Furthermore, several studies have reported that *C. auris* can colonizes the skin and act as a nidus of infection in hospital settings. [51-54] Given these reports, exploring the potential role of efinaconazole in the treatment of resistant *C. auris* is a worthwhile strategy.

Keeping in mind that molds can also cause superficial fungal infections [55], we tested the effect of efinaconazole against three of the common mold species reported to affect the skin (*Scedosporium, Fusarium*, and *Scopulariopsis*) compared with other antifungals. Although all isolates exhibited reduced susceptibility against the tested antifungals, efinaconazole had the highest antifungal activity. In agreement with this observation, in an additional study that included 21 *Fusarium* species, efinaconazole demonstrated an inhibitory effect at a concentration ranging between 0.031 – 2 μg/mL. On the other hand, itraconazole failed to suppress the growth of *Fusarium* as indicated by an MIC range of 16–>32 μg/mL. [55] [45]

It is important to note that our results were generated using 50% growth inhibition as an endpoint since 100% cidal activity was not observed. However, although there was a difference in the endpoint inhibition between our study and previous reports (i.e., 50% vs
100%), our data demonstrates that efinaconazole has more potent in vitro activity against *Fusarium* spp. compared to itraconazole and terbinafine.

Finally, it is important to mention that the data generated in our study was based on *in vitro* experiments. Thus, investigating the effect of efinaconazole in *in vivo* setting and human subjects will provide more insight into the clinical activity of this antifungal.

**Conclusion**

Efinaconazole demonstrated high *in vitro* activity against a wide range of dermatophytes and non-dermatophyte (i.e., mold and yeast) organisms commonly implicated in onychomycosis and tinea infections. Increased activity against a number of isolates that exhibited elevated MIC values against terbinafine and itraconazole was observed, suggesting efinaconazole may be a more efficacious treatment for more resistant organisms. In this same manner, efinaconazole has also shown good activity against *C. auris*, where a large percentage of strains are known to exhibit high levels of resistance to antifungal treatments, warranting further investigation of efinaconazole as a potential therapeutic candidate for *C. auris*.
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