Antifungal Susceptibilities of Paecilomyces Species

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The MICs and minimum fungicidal concentrations (MFCs) of amphotericin B, miconazole, itraconazole, ketoconazole, fluconazole, and flucytosine for 52 isolates of Paecilomyces species were evaluated by the broth microdilution method, largely based on the recommendations of the National Committee for Clinical Laboratory Standards (document M27-A). The fungal isolates tested included 16 P. variotii, 11 P. lilacinus, 9 P. marquandii, 6 P. fumosoroseus, 4 P. javanicus, and 2 P. viridis isolates and 1 isolate of each of the following species: P. carneus, P. farinosus, P. fulvus, and P. niveus. The MFCs and the MICs at which 90% of isolates were inhibited (MIC90s) for the six antifungal agents were remarkably high; the MIC90s indicated that amphotericin B, miconazole, itraconazole, and ketoconazole had good activities, while fluconazole and flucytosine demonstrated poor efficacy. The ranges of the MICs were generally wider and lower than those of the MFCs. There were significant susceptibility differences among the species. All species with the exception of P. variotii were highly resistant to fluconazole and flucytosine; P. variotii was susceptible to fluconazole. Amphotericin B and the rest of the azoles showed good activity against P. variotii, while all the antifungal agents assayed showed low efficacy against P. lilacinus.

In recent years, opportunistic fungal infections have increased substantially, and the species of the genus Paecilomyces are emerging as the cause of a variety of infections in humans (4, 5, 14). Paecilomyces comprises numerous saprobic species, which are regularly isolated from soil and air and some of which are also rather common in food, paper, and other materials. P. variotii, a thermotolerant species often isolated from hay, is probably the most common. Apart from this species, five more species have been reported as producing opportunistic infections in humans (18). Nowadays, the number of reported cases of illness caused by the members of this genus has passed 60, ranging in severity from nail infections to fatal endocarditis. In approximately 90% of the patients some predisposing factor to infection was found: transplants, cardiac surgery, diabetes, trauma, prostatic implants, leukemia, peritoneal dialysis, corticosteroid treatments, etc. The proper treatment for such infections is not yet well established; amphotericin B, miconazole, itraconazole, and ketoconazole are emerging as the cause of a variety of infections in humans and has been used alone or in combination with other drugs, although it has been marketed as the most suitable drug for these infections in humans and has been used alone or in combination with other drugs, although it has a failure rate of about 40%. There is very little information about the in vitro activities of antifungal agents against the Paecilomyces species. The widest-ranging study evaluated the susceptibilities of four strains of P. lilacinus to five antifungal drugs (13), while all the others tested the susceptibility of only one strain. Therefore, the main objective of this study was to evaluate the in vitro antifungal susceptibilities of a certain number of Paecilomyces sp. strains in order to obtain consistent data which could be used as a guide for in vivo treatments. The influence of incubation time on the MICs was also evaluated.

MATERIALS AND METHODS

Test organisms. The 52 Paecilomyces sp. isolates evaluated in this study included 16 P. variotii, 11 P. lilacinus, 9 P. marquandii, 6 P. fumosoroseus, 4 P. javanicus, and 2 P. viridis isolates and 1 isolate each of P. carneus, P. farinosus, P. carneus, P. fulvus, and P. niveus. P. variotii ATCC 36257 was included as the quality control.

Antifungal agents. The following six antifungal agents were used: amphotericin B (E. R. Squibb & Sons, Barcelona, Spain), flucytosine (Hoffmann-La Roche, Basel, Switzerland), fluconazole (Pfizer, Madrid, Spain), ketoconazole (Roig-Farma, Barcelona, Spain), miconazole (Roig-Farma, Barcelona, Spain), and itraconazole (Janssen Pharmaceutica, Beerse, Belgium). Fungizone and Diflucan, the commercial intravenous preparations of amphotericin B and fluconazole, respectively, were used as stock solutions. Antifungal solutions were prepared as described previously (34).

Broth microdilution method. Broth microdilution testing was performed in sterile, 96-well microplates with RPMI 1640 medium. The method that was established has been described in a previous article (34). Aliquots of 100 μl of the drug dilutions were inoculated into the wells. The microplates were stored at −70°C until they were used. The isolates were maintained at 4°C as pure cultures on oatmeal agar (OMA) slants covered with mineral oil. For each experiment, the strains were subcultured onto the OMA slants at 30°C for 15 days, and the inoculum was prepared by scraping the surface of the sporulated fungi with a loop and directly suspending the fungal material in sterile distilled water. The organisms in the resulting suspension were manually counted with a hemacytometer, and the suspension was found to contain 2.5 x 105 conidia. The hemacytometer counts were verified by serial dilution on OMA plates. The conidia were diluted in sterile distilled water to produce a working suspension, which was 1 x 107 to 5 x 107 conidia per ml. The final test drug concentrations were 0.03 to 16 μg/ml for amphotericin B, miconazole, itraconazole, and ketoconazole, 0.125 to 64 μg/ml for fluconazole, and 0.25 to 128 μg/ml for flucytosine. The microplates were incubated without agitation at 25°C, and readings were made after 48 and 72 h.

The amphotericin B MICs were defined as the lowest drug concentration with which there was a complete absence of growth. The azole and flucytosine MICs were defined as the lowest drug concentrations that gave only a slight growth corresponding approximately to 25% of the growth control.

The minimum fungicidal concentrations (MFCs) were determined by plating 100 μl from each negative well and from the positive growth control well onto drug-free OMA, with subsequent incubation at 25°C for 48 h or until subcultures started to grow from the growth control well. The MFC was defined as the lowest concentration of drug from which subcultures were negative or which yielded fewer than two colonies, representing a killing factor of 99%.

Data analysis. The geometric mean MICs and MFCs were calculated for those species for which we tested at least four isolates (P. variotii, P. lilacinus, P. marquandii, P. fumosoroseus, and P. javanicus). The MICs and MFCs at which 50 and 90% of the strains are inhibited (MIC50 and MIC90, respectively, and MFC50 and MFC90, respectively) and the MIC and MFC ranges were calculated for all isolates tested. For each antifungal agent, MIC and MFC results were included in the analysis. The high off-scale MICs and MFCs (e.g., ≥16 μg/ml) were converted to the next highest concentrations (32 μg/ml). The low off-scale MICs and MFCs were left unchanged. When skips (uneven patterns) were present, the MIC endpoint was the highest drug concentration.

Because our MIC distribution values were far from being normal, we used nonparametric methods to compare the in vitro effect of each antifungal agent...
with the three most common species of Paecilomyces (P. variotii, P. lilacinus, and P. marquandii). The rest of the species could not be analyzed because of the small number of strains of each species. The Kruskall-Wallis test was used to determine if the means for the analyzed species were significantly different. When they were, the Mann-Whitney U test was used to study which pairs of species were different. The degree of agreement between the MICs at 48 and 72 h for the six drugs is presented in Table 3. Excellent agreement was shown for fluconazole (k = 1), ketoconazole (k = 0.86), and fluconazole (k = 0.77), good agreement was shown for miconazole (k = 0.75), and poor agreement was shown for amphotericin B (k = 0.39) and itraconazole (k = 0.29).

**RESULTS**

All the isolates of the Paecilomyces spp. tested with the exception of one strain of P. nives produced clearly detectable growth in 48 h. The P. nives isolate required 1 more day. Table 1 presents the MIC<sub>50</sub>, MIC<sub>90</sub>, MFC<sub>50</sub>, and MFC<sub>90</sub> of the six antifungal agents for the 52 strains of Paecilomyces spp. tested. The MIC ranges were generally wider and lower than the MFC ranges. The MIC<sub>50</sub>, MIC<sub>90</sub>, and MFC<sub>50</sub> of the six antifungal agents were remarkably high. The MIC<sub>50</sub> indicated that amphotericin B, miconazole, itraconazole, and ketoconazole have high levels of activity, although, on the contrary, fluconazole and flucytosine demonstrated very poor efficacy.

The geometric mean MICs and MFCs of the antifungal agents for P. variotii, P. lilacinus, P. marquandii, P. fumosoroseus, and P. javanicus are presented in Table 2. In all cases the MFCs were considerably higher than the MICs. The MIC results showed significant differences in susceptibility among the species. All the species with the exception of P. variotii were highly resistant to fluconazole and fluconazole, P. variotii was susceptible to fluconazole. As far as the other antifungal agents are concerned, P. variotii was revealed to be the most susceptible species; the mean MICs of amphotericin B, miconazole, itraconazole, and ketoconazole for this species showed that they had high levels of activity. The MICs of amphotericin B, miconazole, and ketoconazole for P. fumosoroseus and P. javanicus were moderately low. Itraconazole showed efficacy against P. javanicus but not against P. fumosoroseus. All the antifungal agents assayed had poor activity against P. lilacinus and P. marquandii. The only significant difference between these two species was that P. lilacinus was much more resistant than P. marquandii to amphotericin B.

**DISCUSSION**

This is the most extensive study on the antifungal susceptibilities of Paecilomyces spp. performed up to now. We used a broth microdilution method because in general its results agreed with the ones obtained by the broth microdilution method recommended by the National Committee for Clinical Laboratory Standards (document M27-A) (29), as has been repeatedly demonstrated with clinical yeasts (3, 8, 9, 36) and filamentous fungi (10, 33). In a previous comparative study performed with six strains of Paecilomyces spp., there were no significant differences in the MICs obtained by both techniques (unpublished data). For these reasons and because it is more practical and economical than the broth microdilution method, we chose the microdilution method. Moreover, most clinical microbiology laboratories are more familiar with the broth microdilution techniques used for antibacterial agents.

**TABLE 1. Antifungal susceptibilities of 52 isolates of Paecilomyces spp. at 48 h of incubation**

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>MIC (µg/ml)</th>
<th>MFC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.03–&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Miconazole</td>
<td>0.03–&gt;16</td>
<td>1</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.03–&gt;16</td>
<td>2</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.03–&gt;16</td>
<td>2</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0.125–&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>0.25–&gt;128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

**TABLE 2. Susceptibilities of five species of Paecilomyces to six antifungal agents at 48 h of incubation**

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of strains</th>
<th>Amphotericin B</th>
<th>Miconazole</th>
<th>Itraconazole</th>
<th>Ketoconazole</th>
<th>Fluconazole</th>
<th>Flucytosine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC (µg/ml)</td>
<td>MFC (µg/ml)</td>
<td>MIC (µg/ml)</td>
<td>MFC (µg/ml)</td>
<td>MIC (µg/ml)</td>
<td>MFC (µg/ml)</td>
</tr>
<tr>
<td>P. variotii</td>
<td>16</td>
<td>0.08&lt;sup&gt;4&lt;/sup&gt;</td>
<td>13.96</td>
<td>1.04&lt;sup&gt;4&lt;/sup&gt;</td>
<td>24.83</td>
<td>0.07&lt;sup&gt;4&lt;/sup&gt;</td>
<td>6.47</td>
</tr>
<tr>
<td>P. lilacinus</td>
<td>11</td>
<td>10.29&lt;sup&gt;4&lt;/sup&gt;</td>
<td>31.04</td>
<td>6.02</td>
<td>32.06</td>
<td>7.51</td>
<td>31.99</td>
</tr>
<tr>
<td>P. marquandii</td>
<td>9</td>
<td>1.41</td>
<td>8.01</td>
<td>5.03</td>
<td>23.49</td>
<td>5.88</td>
<td>31.99</td>
</tr>
<tr>
<td>P. fumosoroseus</td>
<td>6</td>
<td>0.63</td>
<td>10.09</td>
<td>1.99</td>
<td>21.37</td>
<td>6.35</td>
<td>23.98</td>
</tr>
<tr>
<td>P. javanicus</td>
<td>4</td>
<td>0.84</td>
<td>2.37</td>
<td>2.37</td>
<td>15.99</td>
<td>1.66</td>
<td>15.99</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly different from those for P. lilacinus and P. marquandii (P < 0.05).
<sup>b</sup> Significantly different from that for P. marquandii (P < 0.05).
Paecilomyces \textit{vitro} susceptibilities of P. variotii and P. lilacinus, show very clear differences in their in vitro susceptibilities to the currently used antifungal agents. Of the six drugs tested, only fluconazole showed poor activity against \textit{P. variotii}, and \textit{P. variotii} was the only species susceptible to fluconazole. Only a few clinical isolated of \textit{P. variotii} have been tested by various investigators (5, 7, 15, 22, 23, 27, 31, 35, 37, 38). More than one strain was tested in only two cases: three strains were tested by Marzek et al. (22) and two strains were tested by Chan et al. (5). The techniques used were variable but showed that isolates were susceptible to both fluconazole and itraconazole whenever they were tested. Only one strain was defined as being resistant to amphotericin B (27). Miconazole was tested with eight isolates, and four of the strains were resistant (5, 22). Ketoconazole was assayed with nine isolates, and two were observed to be resistant (27, 31). Fluconazole was tested with seven isolates and six of them were resistant (5, 7, 22).

There is less information about the antifungal susceptibility of the other common species, \textit{P. lilacinus}. In our study, ketoconazole was the only antifungal agent that had moderate activity against this species. Seven strains were previously tested by different investigators (4, 13, 26, 30, 43). One group of investigators tested three strains (13), and the rest tested only one strain each. They reported resistance to amphotericin B, fluconazole, and fluconazole and susceptibility to ketoconazole, miconazole, and clotrimazole; one of the three strains tested was resistant to itraconazole. The results of clinical treatments with amphotericin B have been contradictory. The drug was not effective in three patients (1, 19, 30) and gave good results in five patients (11, 21, 25, 39, 41). Jade et al. (19) described one case of cellulitis caused by \textit{P. lilacinus} that could not be cured with amphotericin B but which was resolved when fluconazole was added to the therapy. A potential synergism or additivism between these drugs (32) may explain the result.

Apart from the species mentioned above, only one strain of \textit{P. marquandii} has been tested in vitro, and it was resistant to amphotericin B and fluconazole and was susceptible to miconazole (16). There were no previous data about the in vitro susceptibility of \textit{P. javanicus}, even though this species has been responsible for two cases of endocarditis (2, 17). Our study included four strains of \textit{P. javanicus}, for which the MICs of amphotericin B, miconazole, itraconazole, and ketoconazole were low. However, the two patients with endocarditis died, despite treatment with amphotericin B.

Endocarditis is among the most severe infections caused by \textit{Paecilomyces} spp., and the mortality rate among patients with \textit{Paecilomyces} endocarditis is high. In total seven cases of endocarditis have been reported, five of which (15, 20, 23, 40, 42) were caused by \textit{P. variotii} and two of which (2, 17) were caused by \textit{P. javanicus}. All seven patients died, and they had all been treated with amphotericin B.

Peritonitis is another relatively common infection caused by \textit{Paecilomyces} spp., and \textit{P. variotii} complicated continuous ambulatory peritoneal dialysis in nine patients. Four patients received amphotericin B intravenously (6, 22, 28), and the rest were treated only with oral antifungal drugs (5, 7, 22). Surprisingly, one patient was cured after being treated only with fluconazole (7), despite the in vitro resistance of his isolate. All patients were cured, but the peritoneal catheter had to be removed before the fungus was eradicated.

Miconazole was efficient in vitro and was successfully used on two occasions, one of which was for a patient with cellulitis caused by \textit{P. marquandii} (16) and the other was for a patient with keratitis caused by \textit{P. lilacinus} (13). Nowadays, this drug is hardly used because of its known side effects.

Itraconazole has been used very little against \textit{Paecilomyces} spp. infections, but on the basis of its good in vitro response against \textit{P. variotii} and \textit{P. javanicus}, it may be worthy of use in patients with severe cases of infection, such as endocarditis, when other drugs have failed.

Only rarely have the MFCs of antifungal drugs for filamentous fungi been determined, and this could be a more predictive parameter (24). It is probable that the MFCs would have shown a higher degree of correlation than the MICs for the isolates mentioned above.

Incubation time had little or no effect on the MICs of fluconazole, fluconazole, ketoconazole, and miconazole. In contrast, amphotericin B and itraconazole MICs showed considerable differences when the two incubation times were compared. They were higher at 72 h. This behavior has also been shown for amphotericin B with other filamentous fungi (34). The low degree of stability of amphotericin B during incubation may account for this difference, but in the case of itraconazole, it is more difficult to explain.

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### REFERENCES
