Aspergillus versicolor as cause of onychomycosis: report of 12 cases and susceptibility testing to antifungal drugs


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Abstract

Background Onychomycoses caused by opportunistic moulds are not well understood, and many are due to Scopulariopsis brevicaulis and other species. Aspergillus versicolor is not documented as an etiological agent in most studies. We have found an increasing prevalence of this species which is involved in 5.8% of all fungal infections of toe nails.

Objective To study the clinical and mycological characteristics of the onychomycosis caused by A. versicolor and the in vitro susceptibility of this mould to antifungal agents.

Results Onychomycosis due to A. versicolor is mainly seen in people over 60 and presents with chronic involvement of the big toe nails. Predisposing factors are not always present and the infection does not respond to conventional topical antifungals. In vitro, A. versicolor has been shown to be resistant to griseofulvin and fluconazole as well as to amphotericin B, whereas MICs for itraconazole and ketoconazole are variable but within a range of 0.50–4.0 μg/ml; on the contrary, MICs for terbinafine are very low (<0.125 μg/ml).

Discussion Aspergillus versicolor could be considered as an emergent pathogen causing toenail onychomycosis. Local treatment seems not to be effective. Of the various systemic antifungal agents studied terbinafine appears to be the most effective in treating onychomycosis.

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Keywords: Aspergillus versicolor; Onychomycosis; MIC; Terbinafine; Azoles; Griseofulvin; Amphotericin B

1. Introduction

Nail infections caused by opportunistic fungi represent, in our environment, around 7.6% of all cases of onychomycosis, the most common causative agent being Scopulariopsis brevicaulis, found in 4.8% of 354 samples studied between 1986 and 1993 [1]. During the same study nine cases of toenail infections were recorded caused by Aspergillus versicolor, the second fungus in terms of frequency. It is worthwhile noting the high incidence of A. versicolor in nail lesions, as this is not a common laboratory contaminant species nor has its presence been described in aerobiological or environmental studies published; at most it has a very low and sporadic incidence in human fungal infection all year round [2,3].

Together with other species of the genus Aspergillus, such as A. flavus and A. fumigatus, A. versicolor is frequently found in stored cereals, hay, cotton, cheese, meat and other foods in a state of decomposition, as well as in various types of soil. A. versicolor has been classified within the section versicolor in the subgenus
Nidulantes [4]; also, some potentially toxic products, such as 6-methoxysterigmatocystine and aversine, an antraquinone derivative, as well as the yellow-orange pigment known as versicolorine [5], have been isolated from its metabolites although their significance as pathogenic agents in infectious processes is practically unknown.

In an earlier publication we described one case of bilateral toenail onychomycosis due to Aspergillus versicolor in an elderly patient with a peripheral circulatory disorder [6]. Since then, we have had the opportunity to examine new cases of nail infections: a relationship between A. versicolor and onychomycosis has been reported in the related species A. sydowi or A. versicolor itself [7] although neither the clinical nor the epidemiological characteristics were given.

The purpose of this paper is to present a series of 12 new cases of onychomycosis due to A. versicolor, evaluating their clinical and epidemiological characteristics. Furthermore, we present the results obtained from the in vitro sensitivity study of this fungus against systemic antifungals which could be useful in the treatment of this condition.

2. Material and methods

Between 1988 and 1995, 205 patients with lesions in the toenails which were clinically compatible with onychomycosis were positive for fungi. The species isolated in 12 patients (5.8%) were identified as Aspergillus versicolor in two or more successive samples; Scopulariopsis brevicaulis was isolated in 7.5% cases, Acremonium spp. and Fusarium solani in two (1%), while dermatophytes accounted for the remaining 67.7% and yeasts 18%.

All samples were planted in Petri plates with Sabouraud dextrose agar with chloramphenicol (Difco, Detroit, MI, USA) and added in the same medium cycloheximide. In each plate, the nail flakes were distributed in 12–20 inoculum spots. Part of the material was used for fresh microscopic examination with 40% potassium hydroxide.

In order to confirm the etiological role of a mold as the causative agent of an ungual lesion, we followed the criteria proposed by English et al. [8]; thus, in the 12 cases where A. versicolor was isolated, culture was repeated two or more times to confirm the result. All patients selected were positive in direct microscopy and A. versicolor was the only microorganism isolated from the nail. A case card was completed for each patient where all relevant data, specifically age, sex, origin, description of lesions and nails affected, time of evolution, associated conditions and treatments instituted, was recorded.

The isolated strains were identified using the methods described by Raper and Fenell [5] and Hoog and Guarro [9], their identification being confirmed in the Mycology Laboratory, Microbiology Department, Medicine School, Universitat Rovira i Virgili, Reus. All isolates were maintained by periodic replanting in Czapek agar medium in our laboratory’s collection, until they were used for in vitro studies of sensitivity to antifungals, at which time one strain per patient was replanted, after ensuring that it was a pure A. versicolor culture. Aspergillus versicolor CECT (Spanish Collection of Standard Strains) 2814 was used as the reference control strain.

Following the method recommended by the Antifungal Standardization Subcommittee of the NCCLS [10], the minimal inhibitory concentrations for each strain were determined in microplates using a RPMI + 20% glucose medium. The inoculum was prepared using the method of Espinel-Ingroff et al. [11] and the antifungals studied were amphotericin B, itraconazole, fluconazole, ketoconazole, griseofulvin and terbinafine; the product was supplied as a powder by the manufacturing laboratories (Janssen, Ketocanazole and Itraconazole; Pfizer, Fluconazole; Squibb, Amphotericin B; Sandoz, Terbinafine). Griseofulvin was obtained from Laboratorios Sigma (G 4753).

The MIC was determined 72 h after incubation at 30°C by visual reading on an inverted mirror, making a spectrophotometric reading also at 405 nm. The MICs end-points were defined as the lowest drug concentration which resulted in 80% reduction of fungal growth compared with the drug free control.

All determinations were done in duplicate.

3. Results

3.1. Clinical characteristics

Six of the twelve patients were male and six female. Except for two cases, they were all from the Barcelona
Table 1

Cases of onychomycosis due to *Aspergillus versicolor* diagnosed between 1988 and 1995

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Nail affected</th>
<th>Microscopic examination</th>
<th>Evolution (years)</th>
<th>Associated conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>Male</td>
<td>83</td>
<td>VI</td>
<td>Hypha + conidia</td>
<td>20</td>
<td>Heart disease</td>
</tr>
<tr>
<td>JP</td>
<td>Male</td>
<td>60</td>
<td>I,VI</td>
<td>Hypha + conidia</td>
<td>3</td>
<td>Trauma</td>
</tr>
<tr>
<td>LZ</td>
<td>Female</td>
<td>33</td>
<td>VI</td>
<td>Hypha</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>JPA</td>
<td>Male</td>
<td>54</td>
<td>I,VI</td>
<td>Hypha</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>EG</td>
<td>Female</td>
<td>60</td>
<td>I,VI</td>
<td>Hypha + conidia</td>
<td>8</td>
<td>Diabetes</td>
</tr>
<tr>
<td>GP</td>
<td>Female</td>
<td>60</td>
<td>I,II,III</td>
<td>Hypha</td>
<td>8</td>
<td>Diabetes, arthritis</td>
</tr>
<tr>
<td>ISM</td>
<td>Male</td>
<td>57</td>
<td>I,VI</td>
<td>Hypha</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CER</td>
<td>Female</td>
<td>69</td>
<td>I,VI</td>
<td>Hypha</td>
<td>20</td>
<td>Venous insuff.</td>
</tr>
<tr>
<td>EMZ</td>
<td>Male</td>
<td>70</td>
<td>I,VI</td>
<td>Hypha</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>FBP</td>
<td>Female</td>
<td>58</td>
<td>I,VI</td>
<td>Hypha</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>CMS</td>
<td>Female</td>
<td>59</td>
<td>I,VI,III</td>
<td>Hypha</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>DVR</td>
<td>Female</td>
<td>63</td>
<td>I,VI</td>
<td>Hypha</td>
<td>?</td>
<td></td>
</tr>
</tbody>
</table>

Time of evolution of nail lesions could be determined in 10 patients and it was longer than 3 years in most of them; one case had a history of more than 20 years.

Most patients had undergone treatment with topical antifungals (mostly azole compounds) in one or more occasions, without clinical improvement.

Associated conditions were found in four patients, with a predominance of diabetes and venous circulatory insufficiency in the lower limbs. One patient had a history of significant local trauma prior to development of infection.

Samples from the internal plateau of the nail were easily obtained by scraping with a sterile scoop or scalpel; direct examination was positive in all cases. In nine cases there were hyphal fragments, and hypha together with conidial structures in samples from three patients. In two cases, nail biopsy was performed and examination showed the presence of PAS-positive branched hyphae (Fig. 2).

### 3.2. In vitro sensitivity to antifungals

MICs were highly variable depending on the antifungal under test (Table 2). Griseofulvin and fluconazole showed the highest MICs, thus it was assumed that all strains were resistant to these antifungals. In the case of amphotericin B, seven strains had MICs higher than 64 μg/ml and the rest always showed values >4 μg/ml.

MICs for ketoconazole and itraconazole were
similar, although some strains were more susceptible to itraconazole; on the contrary, susceptibility to terbinafine was very high since the highest MIC was 1 µg/ml and seven strains had values below 0.125 µg/ml.

4. Discussion

Most fungal nail infections affecting the feet correspond to unguium tinea, with *Trichophyton rubrum* and *T. mentagrophytes* as the main etiological agents [12], but is not uncommon either to find in the affected nails yeasts of the *Candida* genus, specifically *C. parapsilosis*, although in some cases their etiological role is debatable. The same is true of some mycelial fungi such as *Fusarium, Acremonium* and *Aspergillus*. There is merit in using English's proposals [8] when it comes to considering whether these fungi are pathogenic or not.

In the 12 patients analyzed in this study, which represented 5.8% of the total onychomycosis of the feet diagnosed, *A. versicolor* was isolated in cultures of several samples taken on different days, thus fulfilling the criteria that define this as a mycosis. The first case detected in our environment was a male patient with onychogryphosis and peripheral circulatory insufficiency from whom *A. versicolor* was isolated on three occasions, and it was also isolated from the toenails after removal, with histological verification of invasion by filaments from this species [6]; since then our laboratory has had more experience in identifying this species in cases of onychomycosis, and in determining their clinical characteristics. Nail lesions caused by *A. versicolor* were indistinguishable from those produced by other moulds and dermatophytes, especially when total dystrophy was observed.

In the series described in this report, a similar incidence was found for both sexes with an average age of 60.5 years (33–83 years). The only nails affected were toenails, primarily the big toe, as often occurs in almost all cases of onychomycosis by opportunistic molds [12] if we exclude *Scybalidium/Hendersonula* spp that also affects fingernails [13]. Most patients studied (nine cases) presented with bilateral involvement.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ampho B</th>
<th>Griseof</th>
<th>Terbinaf</th>
<th>Itracon</th>
<th>Flucon</th>
<th>Keto</th>
</tr>
</thead>
<tbody>
<tr>
<td>4456</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>0.25</td>
<td>1</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>4484</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&lt;0.125</td>
<td>2</td>
<td>&gt;64</td>
<td>4</td>
</tr>
<tr>
<td>4631</td>
<td>8</td>
<td>&gt;64</td>
<td>&lt;0.125</td>
<td>2</td>
<td>64</td>
<td>4</td>
</tr>
<tr>
<td>4681</td>
<td>16</td>
<td>&gt;64</td>
<td>&lt;0.125</td>
<td>4</td>
<td>&gt;64</td>
<td>4</td>
</tr>
<tr>
<td>92.288</td>
<td>4</td>
<td>&gt;64</td>
<td>1.0</td>
<td>0.50</td>
<td>&gt;64</td>
<td>2</td>
</tr>
<tr>
<td>95.041</td>
<td>4</td>
<td>&gt;64</td>
<td>&lt;0.125</td>
<td>2</td>
<td>64</td>
<td>4</td>
</tr>
<tr>
<td>95.764</td>
<td>16</td>
<td>&gt;64</td>
<td>0.25</td>
<td>4</td>
<td>&gt;64</td>
<td>4</td>
</tr>
<tr>
<td>95.427</td>
<td>8</td>
<td>64</td>
<td>0.125</td>
<td>2</td>
<td>&gt;64</td>
<td>2</td>
</tr>
<tr>
<td>95.426</td>
<td>16</td>
<td>&gt;64</td>
<td>1.0</td>
<td>2</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>95.131</td>
<td>8</td>
<td>&gt;64</td>
<td>0.25</td>
<td>2</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>95.210</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>0.50</td>
<td>1</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>CETC 2814</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>0.50</td>
<td>1</td>
<td>&gt;64</td>
<td>1</td>
</tr>
</tbody>
</table>
Fig. 3. Pure culture of Aspergillus versicolor on agar-Sabouraud with cloramphenicol.

Only in one of the five patients, was a history of significant toenail trauma (case no. 2) documented which appeared to have been the predisposing factor; in the other four cases underlying conditions were present that have been described previously as favouring the development of nail infections. These conditions were diabetes and circulatory disorders, which are in general the same as those described for other onychomycoses [14]. The absence of local or general factors that favour onychomycosis in most patients studied, would support a primary pathogenic potential for this species in the healthy nail; however, considering the prolonged evolution of this disease, more than 3 years in nearly all patients, and also the multiple antifungal treatments applied, one cannot rule out the possibility that the primitive nail lesion was caused by a dermatophytic fungus, as has been proposed by several authors [12]; in such cases secondary colonization by Aspergillus would be possible.

Mycological diagnosis of infection by Aspergillus was suspected in two cases where direct microscopic examination showed large quantities of conidia, together with fragments of hyphae. Since the characteristics of these hypha did not differ from those seen in the unguium tinea, the definite diagnosis can only be confirmed by mycological culture.

Repeated isolation in pure culture of a considerable number of colonies (Fig. 3) warrants the diagnosis onychomycosis due to A. versicolor. Nail invasion was histopathologically confirmed in the two cases where it was possible to conduct a biopsy, and where no specific morphological features were seen in the hyphae.

Therapeutic failure was documented in all patients since all had received local treatment mainly with topical azoles, for years or months prior to cultures with no noticeable improvement. The poor sensitivity of the Aspergillus fungi to the antifungals used in the treatment of onychomycosis encouraged studies of their in vitro susceptibility using a standardised method based on NCCLS recommendations [10].

Although amphotericin B is not used in the treatment of this type of mycosis, it was taken as the reference antifungal; also the possibility of A. versicolor causing systemic infections that could be treated with this polyene cannot be ruled out.

It is not surprising that all strains, including the one used as reference, are resistant to griseofulvin and fluconazole since these antifungals are not active against Aspergillus fumigatus [15,16], and although there is no specific data available on A. versicolor, it can be assumed that the absence of activity will also be the rule for this species.

When itraconazole was evaluated, only two strains showed MICs higher than 2 μg/ml; this data coincides with that from Espinel Ingroff et al. [11], for 10 strains of A. fumigatus in which MIC values ranged from 0.063–2 μg/ml, even though these values are higher than those found for A. flavus in the top range of the MIC which was 0.13 μg/ml. The range for MIC90 described by Van Cutsem et al. [17], however, was much higher for species such as A. niger (10–100 μg/ml).

The range found for ketoconazole in A. versicolor was lower than that described for A. fumigatus (4–16 μg/ml) [11,18].

With regard to amphotericin B, there is no information on A. versicolor. The most commonly studied species within the Aspergillus genus have been A. fumigatus and A. flavus, with MICs usually in the range from 0.1 to 1.0 μg/ml, and therefore it is surprising that all A. versicolor strains have presented with MICs higher than 4 μg/ml, and three of them equal to or higher than 64 μg/ml. According to these results all strains studied are resistant to this polyene.

The most remarkable finding in this study was the extreme susceptibility of all strains to terbinafine, with
MICs in four strains lower than the maximum dilution of the antifungal taken as the starting point in this study (0.125 μg/ml). Although there is no data on A. versicolor, the high sensitivity of Aspergillus species to this antifungal is well-known but the MICs ranges described by Clayton were less constant than those seen in this study [19]. The results obtained show MIC values lower than those described for A. flavus, A. fumigatus and A. niger [20].

This data suggests that itraconazole and terbinafine (the latter has in vitro fungicidal action) [21] could be used in the oral treatment of fungal infections caused by A. versicolor. This assumption should be confirmed in further therapeutic trials. The administration of terbinafine in a few cases of systemic aspergillosis for ‘compassionate’ programs has been recorded [22]; Schiraldi et al. [23] recently reported favourable therapeutic results with good tolerance of treatment in three immunocompetent patients with chronic aspergillosis empyema (one case), and chronic necrotizing aspergillosis in the other two, all of them caused by A. fumigatus. This data allows us to propose the alternative therapy of onychomycosis caused by Aspergillus versicolor with terbinafine, given the accumulation of this antifungal in the nail tissue and its fungicidal effect [20,24].

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References

