# *In vitro* antifungal susceptibility of nondermatophytic keratinophilic fungi

**Josep Guarro[1], Isabel Pujol[2], Carmen Aguilar[1] and Montserrat Ortoneda[1]**

*[1]Unitat de Microbiologia, Facultat de Medicina, Universitat Rovira i Virgili and [2]Laboratori de Microbiologia, Hospital Universitari de Sant Joan de Reus, Reus, Tarragona, Spain*

*Summary* Nondermatophytic keratinophilic fungi like *Scytalidium* spp. and *Chrysosporium* spp. have been associated with superficial skin infections in humans. However, there have also been reports of more severe infections both localized and disseminated caused by these fungi. The best way to treat such infections has not yet been defined. We have evaluated the *in vitro* activity of amphotericin B, flucytosine, fluconazole, ketoconazole, miconazole and itraconazole against 29 strains of representative species of these two genera (17 strains of *Scytalidium* spp. and 12 strains of *Chrysosporium* spp.), by adapting the method of the National Committee for Clinical Laboratory Standards for testing filamentous fungi (M38-P). Amphotericin B and miconazole showed a very good activity against both genera (all isolates were susceptible to both drugs). Ketoconazole, fluconazole and itraconazole showed a better activity against *Chrysosporium* (all strains were susceptible to ketoconazole and fluconazole and 25% resistant to itraconazole) than against *Scytalidium*. 7.69% of the strains of *Scytalidium* were resistant to ketoconazole, 15.38% were resistant to fluconazole and 62.50% to itraconazole. Flucytosine was more active against *Scytalidium* (23% of the strains resistant) than against *Chrysosporium* (all strains resistant). From *in vitro* data and the results of some clinical treatments, we conclude that amphotericin B should be the drug used in the treatment of severe infections by *Scytalidium* and *Chrysosporium* species.

*Key words* Antifungal susceptibility, Microdilution method, *Scytalidium*, *Chrysosporium*

The infections caused by dermatophytes are probably the most common communicable diseases affecting humans. However, other keratinophilic fungi are also occasionally reported as etiologic agents of human infections. Among them *Scytalidium* spp., *Scopulariopsis* spp. and *Chrysosporium* spp. are relatively frequent [1]. Most *Scytalidium* species are saprophytes on wood or other plant material. *Chrysosporium* spp. live on remains of hairs and feathers in soil, and the "microconidia" present in most of the *Microsporum* and *Trichophyton* cultures are also typical *Chrysosporium*. *Scopulariopsis* spp. are common laboratory contaminants that are also frequently isolated from soil, food, paper and other materials. The most relevant pathogenic species from the above-mentioned genera is *Scytalidium dimidiatum,* also known as *Nattrassia mangiferae, Hendersonula toruloidea* and more recently considered to be indistinguishable from another close species, *S. hyalinum* [2]. Because of their ability to degrade keratin, these species are common causes of nail and skin infections, although more severe infections, both localized and systemic, are also reported [3-5]. In these cases some underlying conditions of the host, such as diabetes, immunosuppression or therapy with corticosteroids are common predisposing factors.

Numerous therapeutic regimens have been used to treat the different types of infections caused by the nondermatophytic keratinophilic fungi but general assessment does not yet exist. *In vitro* antifungal susceptibility tests may be useful but reliable *in vitro* methods need to be developed to detect the activity of antifungal agents that may predict clinical outcome. Several years ago the National Committee for Clinical Laboratory Standards (NCCLS) developed a standardized broth dilution method for the *in vitro* susceptibility testing of yeasts. Interpretative breakpoints have been proposed for some antifungals (fluconazole, itraconazole and flucytosine) on the basis of a comparison of clinical outcome with the *in vitro* results [6]. In the last few years some studies have developed techniques for filamentous fungi [7-14], but their standardization is at a less advanced stage. However, the NCCLS has recently proposed a reference method for testing some species of filamentous fungi (document M38-P)[15], but interpretative criteria for these results have not yet been defined.

In a recently published study we tested several strains of representative species of *Scopulariopsis* [16]. This study tests 29 strains of the most significant species

**Corresponding address:**
Prof. Josep Guarro
Unitat de Microbiologia, Facultat de Medicina,
Universitat Rovira i Virgili, Carrer Sant Llorenç 21,
43201 Reus, Tarragona, Spain
Tel.: +34 977 759 359; Fax: +34 977 759 322
E-mail: umb@astor.urv.es.

of *Scytalidium* and *Chrysosporium* against six antifungal drugs. We have used the NCCLS guidelines [15] but with some modifications because these fungi are not included in them.

***Test organisms.*** A panel of 29 isolates were tested, 17 belonging to *Scytalidium* and 12 to *Chrysosporium* (Table 1). *Paecilomyces variotii* ATCC 36257 was used as the quality control strain (QC).

***Medium.*** Antifungal susceptibility testing was performed using RPMI 1640 medium (GIBCO BRL, Life Technologies, Izasa, Spain) with L-glutamine and without sodium bicarbonate, and buffered at pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; Sigma Chemical Co).

***Antifungal agents.*** The following six antifungal drugs were used: amphotericin B (E.R. Squibb & Sons, Barcelona, Spain), flucytosine (Hoffmann-La Roche, Basel, Switzerland), fluconazole (Pfizer, Madrid, Spain), ketoconazole, miconazole (Roig-Farma, Barcelona, Spain) and itraconazole (Janssen Pharmaceutica, Beerse, Belgium). Fungizone and Diflucan, respectively the commercial preparations of amphotericin B and fluconazole, were used and stock solutions, each at 1,000 µg/ml, were prepared with sterile distilled water. Itraconazole, ketoconazole, miconazole and flucytosine were provided by the manufacturers as standard powders and stock solutions were prepared with the weight adjusted according to the strength of each drug. Stock solutions of itraconazole, ketoconazole and miconazole, at 1,600 µg/ml, were prepared in DMSO (100%) (Sigma Chemical Co, St. Louis, MO, USA). A stock solution of flucytosine, at 5,120 µg/ml, was prepared with sterile distilled water.

***Drug dilutions.*** Drug dilutions were performed by an additive drug dilution scheme, according to the recommendations of the National Committee for Clinical Laboratory Standards (Document M38-P) [15]. Stock drug solutions of amphotericin B, fluconazole and flucytosine were diluted to 10 times the strength of the final drug concentration in RPMI medium (e.g. 160 to 0.3125 µg/ml for amphotericin B, 640 to 1.25 µg/ml for fluconazole and 1,280 to 2.50 µg/ml for flucytosine). Each 10X drug dilution was further diluted 1:5 in RPMI medium to obtain twice the final concentrations. Stock drug solutions of itraconazole, ketoconazole and miconazole were first diluted to 100 times the strength of the final drug concentrations in 100% DMSO (e.g. 1,600 to 3.125 µg/ml) and further diluted 1:50 in medium to obtain 2X drug concentracions. Aliquots of 100 µl of the 2X drug dilutions were inoculated into the wells of a sterile, flat-bottomed 96-well microplate (Greiner Labortechnik, Madrid, Spain) with a multichannel pipette. The first well of each row contained the highest concentration and well 10 contained the lowest concentration. Well 12 of each row was used as the growth control and 100 µl of RPMI medium was dispensed for test amphotericin B, fluconazole and flucytosine and 100 µl of RPMI medium at 1% of DMSO for test itraconazole, miconazole and ketoconazole. The microdilution plates were stored at -20°C until used.

***Inoculum preparation.*** The isolates were maintained at 4°C as pure cultures on potato carrot agar (PCA) slants covered with mineral oil until testing began. For each experiment, strains were subcultured onto PCA slants at 30°C for 15 days and the inoculum was prepared by scraping the surface of the sporulated fungi with a loop and suspending the fungal material in sterile distilled water. The suspension was then filtered once through sterile gauze to remove hyphae, and the resulting conidial suspension was counted with a haemocytometer. The haemocytometer counts were verified by serial dilutions on PCA plates which were incubated at 30°C for 72 h. The conidia suspension was diluted in sterile distilled water to produce a working suspension of 1-5 X $10^6$ conidia/ml and futher diluted 1:50 in RPMI medium to obtain 2X final suspension.

**Table 1**. *In vitro* susceptibilities of *Scytalidium* spp. and *Chrysosporium* spp.

| | AMB | | MICO | | ITRA | | KETO | | FLU | | 5-FC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain[a] | MIC[b] | MFC[b] | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC |
| *S. dimidiatum* CBS 136.77 | 0.12 | 2 | ≤0.03 | 0.25 | ≤0.03 | 0.25 | ≤0.03 | 4 | 0.50 | 2 | 16 | >128 |
| *S. dimidiatum* CBS 137.77 | 0.12 | 1 | 1 | 1 | 2 | >16 | 1 | 1 | 16 | 32 | 16 | 64 |
| *S. dimidiatum* CBS 661.77 | 0.12 | 1 | 8 | >16 | >16 | >16 | - | - | - | - | - | - |
| *S. dimidiatum* CBS 662.77 | 0.12 | 1 | 4 | 8 | >16 | >16 | - | - | - | - | - | - |
| *S. dimidiatum* CBS 637.89 | 0.12 | 1 | 4 | >16 | >16 | >16 | - | - | - | - | - | - |
| *S. dimidiatum* CBS 312.90 | 0.12 | 1 | 0.12 | 0.12 | 0.12 | 0.12 | - | - | - | - | - | - |
| *S. dimidiatum* CBS 131.70 | 0.50 | 4 | 4 | >16 | >16 | >16 | 8 | >16 | 32 | >64 | 16 | >128 |
| *S. dimidiatum* CBS 251.49 | 0.12 | 0.50 | 0.50 | 2 | 0.06 | 0.5 | 0.25 | 0.25 | 4 | >64 | 32 | 128 |
| *S. dimidiatum* CBS 204.33 | 0.06 | 0.50 | 0.50 | 8 | 2 | >16 | 2 | >16 | 8 | 64 | 32 | 64 |
| *S. hyalinum* CBS 545.95 | 0.06 | 0.06 | 1 | >16 | 2 | >16 | 4 | 8 | 32 | >64 | 8 | 16 |
| *S. hyalinum* CBS 200.88 | 1 | >16 | 4 | >16 | >16 | >16 | 16 | >16 | 64 | >64 | 16 | >128 |
| *S. hyalinum* CBS 619.84 | 0.06 | 0.06 | 2 | 2 | >16 | >16 | 8 | >16 | 64 | >64 | 8 | >128 |
| *S. hyalinum* CBS 466.81 | 0.12 | 0.12 | 1 | 2 | 2 | >16 | 4 | 4 | 32 | 32 | 4 | 4 |
| *S. infestans* CBS 161.91 | 1 | 4 | 1 | >16 | 0.12 | >16 | 0.25 | >16 | 8 | >64 | 2 | >128 |
| *S. japonicum* CBS 494.88 | 0.06 | 2 | 1 | 4 | 0.25 | 4 | 0.50 | 0.50 | 16 | 64 | >128 | >128 |
| *S. lignicola* CBS 387.59 | 0.50 | - | - | - | 0.06 | - | 0.50 | - | 16 | - | ≤0.25 | - |
| *S. lignicola* CBS 204.71 | 0.50 | 16 | 2 | 4 | 0.50 | 2 | 1 | 1 | 16 | 64 | ≤0.25 | 16 |
| *C. keratinophilum* CBS 104.62 | ≤0.03 | 0.50 | 2 | 16 | 0.50 | >16 | 0.50 | >16 | 8 | >64 | >128 | >128 |
| *C. keratinophilum* FMR 6030 | 0.50 | 8 | 1 | >16 | 0.50 | >16 | 0.50 | >16 | 8 | >64 | >128 | >128 |
| *C. keratinophilum* FMR 6031 | 0.12 | 16 | 1 | >16 | 0.50 | >16 | 0.25 | >16 | 4 | >64 | >128 | >128 |
| *C. zonatum* CBS 340.89 | 0.50 | 2 | 1 | >16 | 0.50 | >16 | 1 | >16 | 8 | >64 | >128 | >128 |
| *C. zonatum* FMR 6047 | 0.50 | 4 | 1 | >16 | 0.50 | 4 | 0.25 | >16 | 4 | >64 | >128 | >128 |
| *C. zonatum* FMR 6048 | 0.50 | 4 | 1 | >16 | 1 | >16 | 0.50 | >16 | 16 | >64 | >128 | >128 |
| *C. tropicum* CBS 171.62 | 0.12 | 8 | 1 | >16 | 0.50 | >16 | 0.50 | >16 | 8 | >64 | >128 | >128 |
| *C. tropicum* FMR 6065 | 0.50 | 4 | 1 | 16 | 0.50 | 16 | 0.25 | >16 | 2 | 32 | >128 | >128 |
| *C. tropicum* FMR 6066 | 0.25 | 0.50 | 1 | >16 | 1 | 4 | 0.50 | >16 | 8 | 32 | >128 | >128 |
| *A. fulvescens* FMR 6155 | 0.06 | 0.50 | 0.50 | 16 | 0.50 | >16 | 0.50 | >16 | 4 | >64 | >128 | >128 |
| *A. fulvescens* FMR 6156 | ≤0.03 | 2 | 2 | >16 | 1 | >16 | 1 | >16 | 4 | 64 | >128 | >128 |
| *A. fulvescens* FMR 6157 | 0.50 | 2 | 4 | >16 | 0.50 | >16 | 0.50 | >16 | 4 | >64 | >128 | >128 |

[a] CBS, culture obtained from Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; FMR, Faculty of Medicine, Reus, Tarragona, Spain.
[b] µg/ml, AMB, amphotericin B; MICO, miconazole; ITRA, itraconazole; KETO, ketoconazole; FLU, fluconazole; 5-FC, flucytosine.

***Broth microdilution method.*** Each well of the microdilution plates was inoculated on the day of the test with 100 µl of the 2X inoculum suspension. This step brought the drug dilutions and inoculum to the final test concentrations of 16 to 0.0313 µg/ml for amphotericin B, itraconazole, ketoconazole and miconazole, 64 to 0.125 µg/ml for fluconazole, 128 to 0.25 µg/ml for flucytosine and 1-5 X $10^4$ conidia/ml, respectively. Growth control wells were inoculated with 100 µl of the corresponding inoculum suspension. One well of the microdilution plates containing 200 µl RPMI medium was included as the sterility control medium. The QC organism was tested in the same way and included each time a set of isolates was tested. The inoculated microplates were incubated without agitation at 30°C and MICs were read with the aid of a reading mirror as soon as the growth control well became turbid i.e. at 48 h for *Scytalidium* and 96 h for the others. Amphotericin B MIC was defined as the lowest drug concentration at which there was no growth. Azoles and flucytosine MICs were defined as the lowest drug concentrations which produced a reduction in growth of ≥ 75% of the corresponding growth control. The minimum fungicidal concentrations (MFCs) were determined by subculturing 10 µl from each negative well on to PCA plates. Plates were incubated at 30°C for 72 h for *Scytalidium* and five days for *Chrysosporium* and colonies of fungi were counted. MFC was defined as the lowest drug concentration from which subcultures yielded no more than one colony (killing factor of 99%).

***Data analysis.*** The geometric means of MICs and MFCs were calculated for each genera. The MICs and MFCs at which 50 and 90% of the strains are inhibited ($MIC_{50}$ and $MIC_{90}$, respectively, and $MFC_{50}$ and $MFC_{90}$, respectively) and the MIC and MFC ranges were calculated for all the isolates. Off-scale MIC and MFC results were included in the analysis. The high off-scale MICs and MFCs (>16, >64 and >128 µg/ml) were converted to the next highest concentration (32, 128 and 256 µg/ml, respectively). The low off-scale MICs and MFCs (≤ 0.03, ≤ 0.12 and ≤ 0.25 µg/ml) were left unchanged (0.03, 0.12 and 0.25 µg/ml, respectively). When skips (uneven patterns) were present, the MIC and MFC endpoints were the highest drug concentrations.

Table 1 shows the MIC and MFC values of the six antifungals tested against 29 strains of filamentous fungi. The order of activity of the six antifungals examined in this study was: amphotericin B > miconazole > ketoconazole > itraconazole > flucytosine > fluconazole against *Scytalidium* isolates; and amphotericin B > ketoconazole > itraconazole > miconazole > fluconazole > flucytosine against *Chrysosporium* isolates (Table 2). For comparison purposes we have used the interpretative criteria for resistance published by Sutton *et al*. [17], who considered the following breakpoints: ≥ 2 µg/ml for amphotericin B; ≥ 1 µg/ml for itraconazole; ≥ 16 µg/ml for miconazole; ≥ 16 µg/ml for ketoconazole; ≥ 64 µg/ml for fluconazole and ≥ 32 µg/ml for flucytosine. According to this criterion and on the basis of MIC results, amphotericin B and miconazole showed a very good activity against *Scytalidium* spp. and *Chrysosporium* spp. All the isolates of both genera were always sensitive to both antifungal drugs. Ketoconazole, fluconazole and itraconazole showed a better activity against *Chrysosporium* (all strains were sensitive to ketoconazole and fluconazole and 25% were resistant to itraconazole) than against *Scytalidium* (the resistance of the strains tested against the same antifungals was 7.69, 15.38 and 62.50%, respectively). On the other hand, flucytosine was more active against *Scytalidium* (23% of the strains were resistant) than against *Chrysosporium* (all strains were resistant). In general, the MFCs of the six antifungals were higher than the corresponding MICs. The $MFC_{50}$ indicated that only amphotericin B, miconazole and ketoconazole have good lethal efficay against *Scytalidium* isolates. However, fluconazole and flucytosine against of *Scytalidium* and the six antifungal agents against *Chrysosporium*, demonstrated very poor lethal activity (Table 2).

*Scytalidium dimidiatum* is a nondermatophitic keratinophilic fungus which is a common cause of human disease. Although infections caused by *Scytalidium dimidiatum* are usually superficial, at least 12 cases of deep infections due to this fungus have so far been described. Approximately half of these patients were diabetic [3-5] (Table 3). *In vitro* antifungal susceptibilities of the clinical isolates was determined in only four of them [3,5,18,19]. In three cases, *in vitro-in vivo* correlation was performed [3,5,18]. Amphotericin B was effective in six of the seven cases in which it was used. However, in only two cases this antifungal was applied alone [20,21]. A combination of amphotericin B and topical clotrimazole was effective in a steroid-dependent diabetic man with a subcutaneous infection in the arm [3]. Treatment with amphotericin B followed by ketoconazole was also effective in a diabetic patient with a maxillary sinus infection [22] and in an HIV-positive patient with skin lesions, lymphangitis and lymphadenitis [5]. A combined therapy of surgical debridement, amphotericin B, nistatyn and itraconazole successfully treated a subcutaneous infection of the foot in an immunosuppressed, diabetic patient [4]. However, a surgi-

**Table 2.** *In vitro* susceptibilities of *Scytalidium* spp. and *Chrysosporium* spp.

| Organisms | Nº isolates | Antifungal agents | MIC[a] Range | MIC$_{50}$ | MIC$_{90}$ | MIC Geometric mean | MFC[a] Range | MFC$_{50}$ | MFC$_{90}$ | MFC Geometric mean |
|---|---|---|---|---|---|---|---|---|---|---|
| *Scytalidium* spp. | 17 | amphotericin B | 0.06 - 1 | 0.12 | 1 | 0.17 | 0.06 - >16 | 1 | 16 | 1.08 |
| | | miconazole | ≤0.03 - 8 | 1 | 4 | 1.13 | 0.12 - >16 | 4 | >16 | 5.16 |
| | | itraconazole | ≤0.03 - >16 | 2 | >16 | 1.61 | 0.12 - >16 | >16 | >16 | 9.44 |
| | | ketoconazole | ≤0.03 - 16 | 1 | 8 | 1.23 | 0.25 - >16 | 4 | >16 | 5.31 |
| | | fluconazole | 0.50 - 64 | 16 | 64 | 14.25 | 2 - >64 | 64 | >64 | 59.56 |
| | | flucytosine | ≤0.25 - >128 | 16 | 32 | 7.94 | 1 - >128 | 128 | >128 | 84.13 |
| *Chrysosporium* spp. | 12 | amphotericin B | ≤0.03 - 0.50 | 0.25 | 0.50 | 0.20 | 0.50 - 16 | 2 | 8 | 2.52 |
| | | miconazole | 0.50 - 4 | 1 | 2 | 1.19 | 16 - >16 | >16 | >16 | 26.92 |
| | | itraconazole | 0.50 - 1 | 0.50 | 1 | 0.59 | 4 - >16 | >16 | >16 | 25.12 |
| | | ketoconazole | 0.25 - 1 | 0.50 | 1 | 0.47 | >16 | >16 | >16 | 32.20 |
| | | fluconazole | 2 - 16 | 4 | 8 | 5.66 | 32 - >64 | >64 | >64 | 95.50 |
| | | flucytosine | >128 | >128 | >128 | 256 | - | - | - | - |

[a] µg/ml

**Table 3.** Deep infections caused by *Scytalidium dimidiatum* in humans described in the literature.

| Reference | Immunosuppression | Localization(s) | *In vitro* susceptibility[a] | Treatment | Outcome |
|---|---|---|---|---|---|
| Mariat *et al.* (1978) | Yes | Skin | ND | AMB | Resolution |
| Dickinson *et al.* (1983) | Not | Skin | 5-FC (S), AMB (S), KETO (S), MICO (S) | Surgical debridement, 5-FC | Resolution |
| Drouhet *et al.* (1983) | Not | Mycetoma | ND | KETO | Non resolution |
| Zaatari *et al.* (1984) | Yes | Skin | MICO (moderate susceptibility) | KETO | Non resolution |
| Migeville (1986) | Not | Maxillary sinus | ND | AMB, KETO | Resolution |
| McGough *et al.* (1992) | Yes | Skin | ND | CLOTRI | Non resolution |
| Al-Rajhi *et al.* (1993) | Not | Eye | ND | Surgical, AMB, MICO, NATA | Non resolution |
| Benne *et al.* (1993) | Yes | Blood, skin | ND | AMB | Resolution |
| Levi *et al.* (1994) | Not | Skin | ND | KETO | Non resolution |
| Rockett *et al.* (1996) | Yes | Skin | ND | Surgical debridement, NIS, AMB, ITRA | Resolution |
| Marriot *et al.* (1997) | Yes | Skin, lymphangitis, lymphadenitis | AMB (S), 5-FC (S), FLU (S), KETO (S). ITRA (R) | AMB, KETO | Resolution |
| Sigler *et al.* (1997) | Yes | Skin | AMB (S), ITRA (S) | AMB, CLOTRI | Resolution |

[a] Abbreviations: AMB, amphotericin B; 5-FC, flucytosine; FLU, fluconazole; ITRA, itraconazole; KETO, ketoconazole; MICO, miconazole; CLOTRI, clotrimazole; NATA, natamycin; NIS, nistatyn; R, resistant; S, susceptible; ND, test not done

cal treatment combined with several antifungals (intraocular amphotericin B, miconazole and topical natamycin) failed to cure a case of endophthalmitis [23]. Monotherapy with different azole derivatives failed to resolve four cases of deep skin infections. Three of these were treated with ketoconazole [19,24,25], while the other was treated with topical clotrimazole [26]. Flucytosine and surgical debridement were effective in the treatments a diabetic man with subcutaneous abscesses. The clinical isolate had been susceptible to this drug *in vitro* [18]. Several azoles, such as clotrimazole, bifonazole, tioconazole, miconazole, ketoconazole, econazole and itraconazole have been used to treat superficial infections and onychomicosis but they have not been very effective, in spite of the susceptibility of these organisms to azoles *in vitro* [3]. In our study, *in vitro* susceptibility testing of several strains of this species demonstrated that all of them were susceptible to amphotericin B and miconazole. Susceptibility to the other azoles and flucytosine was variable. In general, our results agree with those of Sigler *et al*. [3] who reported geometric mean MICs of amphotericin B and miconazole very similar to ours (0.17 and 1.13 µg/ml, respectively). According to the criteria used in our study, all the strains tested by Sigler *et al.* would be susceptible to these two antifungals. On the other hand, there are some differences in susceptibility with the other antifungals tested. Sigler *et al.* described a greater activity of ketoconazole, fluconazole and itraconazole than we have found; all their isolates were susceptible to ketoconazole and fluconazole and 31.82% of the strains were resistant to itraconazole. For flucytosine, our results are better than those of Sigler *et al*., who described a resistance of 42.86% for the strains (23% in our case).

There are numerous species which are morphologically undistinguishable from *Chrysosporium* but which have traditionally been given different names by clinicians and medical mycologists. These are species of *Histoplasma*, *Blastomyces*, *Emmonsia* etc. and the microconidia of the dermatophytes. All are common causes of different types of human infections. However, some species of *Chrysosporium*, different from those mentioned above, are also frequently isolated from clinical specimens [27] and are even involved, although rarely, in human disease. At least five cases have been described in humans. Different organs were affected and the bone was involved in three of them [28-33] (Table 4). Therapy with amphotericin B was effective in two cases of osteomyelitis caused by *C. parvum*, one of which was an immunocompetent patient and the clinical isolate was also susceptible *in vitro* to this drug [28] and the other was an HIV-positive patient with disseminated adiaspiromycosis with bone involvement [32]. Roilides *et al*. [33] described a case of pneumonia and tibia osteomyelitis caused by *C. zonatum* in a patient with chronic granulomatous disease. This patient was initially treated with amphotericin B and responded well. Amphotericin B was discontinued and oral therapy with itraconazole was initiated. However, pneumonia recurred, in association with pericarditis and pleuritis. These manifestations subsided, and there was no recurrence with liposomal amphotericin B therapy. The clinical isolate was susceptible *in vitro* to amphotericin B and resistant to itraconazole. Treatment with amphotericin B and flucytosine failed to resolve a case of invasive infection in a leukemic patient following bone marrow transplant [30,31]. Toshniwal *et al*. [29], described a case of prosthetic-aortic valve endocarditis in a patient with severe atherosclerotic disease. At autopsy, cultures of the vegetations yielded *Chrysosporium* sp., *Aphanoascus* species, the telemorphs of *Chrysosporium* spp., have also been said to cause dermatophytoses in humans [27,34-38] but at present no deep infection has been described as being caused by these fungi. In our study the MICs of antifungals tested against several species of *Chrysosporium* showed that all were susceptible to amphotericin B, miconazole, ketoconazole and fluconazole, and that 25% of the strains were resistant to itraconazole. All strains were resistant to flucytosine. Data for the *in vitro* activities of antifungal agents against *Chrysosporium* species have recently been provided by Wildfeuer *et al*. [39] and Roilides *et al*. (33). The widest study [39] evaluated the activity of voriconazole, itraconazole, ketoconazole and amphotericin B against 10 strains of *C. keratinophilum* and activity was good (geometric mean MICs were 0.08, 0.12, 0.13 and 0.34 µg/ml, respectively). Roilides *et al*. [33] tested the activity of amphotericin B, flucytosine, fluconazole and itraconazole against four iso-

**Table 4.** Deep infections caused by *Chrysosporium* species in humans.

| Reference | Immunosuppression | Localization(s) | Aetiologic agent | *In vitro* susceptibility[a] | Treatment | Outcome |
|---|---|---|---|---|---|---|
| Stillwell *et al.* (1984) | Not | Bone | *C. parvum* | AMB (S), 5-FC (S), MICO (S), Ri (R) | AMB | Resolution |
| Toshniwal *et al.* (1986) | Not | Aortic prosthetic valve vegetation | *Chrysosporium* sp | ND | —— | Non resolution |
| Levy *et al.* (1991) | | | | | | |
| Warnick *et al.* (1991) | Yes | Brain, lungs, liver, kidney, nasal and sinus | *Chrysosporium* sp | ND | AMB, 5-FC | Non resolution |
| Echavarria *et al.* (1993) | Yes | Bone | *C. parvum* var. *parvum* | ND | AMB | Resolution |
| Roilides *et al.* (1999) | Not | Lung, bone | *C. zonatum* | AMB (S), ITRA (R), 5-FC (R), FLU (S) | AMB, ITRA, AMB (liposomal) | Resolution |

[a] Abbreviations: AMB, amphotericin B; 5-FC, flucytosine; FLU, fluconazole; ITRA, itraconazole; MICO, miconazole; Ri, rifampin; R, resistant; S, susceptible; ND, test not done

lates of *Chrysosporium* spp. All isolates were sensitive to amphotericin B (MICs ≤ 0.25 µg/ml) and resistant to flucytosine (MICs > 128 µg/ml). In addition, two isolates were resistant to itraconazole (MICs ≥ 1 µg/ml) and three were resistant to fluconazole (MICs ≥68 µg/ml). Our results are in agreement with these studies and indicate good *in vitro* activity of amphotericin B, ketoconazole and itraconazole against *Chrysosporium* but very poor activity of flucytosine. However, there are some discrepancies about the activity of fluconazole. While our study reports very good activity for this azole (all strains were susceptible), Roilides *et al*. [33] described poor activity (only one strain susceptible).

In conclusion, our *in vitro* results and the data reported in the literature indicate that amphotericin B should be used to treat deep infections by *Scytalidium* and *Chrysosporium.* However, further studies are needed to evaluate the correlation between *in vitro* test results and therapy.

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