

Exophiala spinifera as a cause of cutaneous phaeohyphomycosis: Case study and review of the literature

JOHN E. HARRIS*, DEANNA A. SUTTON†, ADAM RUBIN*, BRIAN WICKES‡, G. S. DE HOOG§ & CARRIE KOVARIK*

*Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, †Department of Pathology, University of Texas Health Science Center, San Antonio, Texas, ‡Department of Microbiology, University of Texas Health Science Center, San Antonio, Texas, USA, and §Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

Exophiala spinifera has been reported as an agent of cutaneous disease 18 times in the literature. Clinical presentations of cutaneous lesions vary widely, including erythematous papules, verrucous plaques, and deep subcutaneous abscesses. The clinical distribution and course of disease are also variable, depending on the age and immune competency of the patient. Histologic appearance occurs in one of two patterns – phaeohyphomycosis or chromoblastomycosis. While *E. spinifera* appears to be susceptible to multiple antimicrobial agents *in vitro*, clinical experience with treatment modalities has been variable. Prior to the availability of sequencing methods, species identification was based on the histopathologic presentation in tissue and morphologic features of the fungus in culture. It is likely that *E. spinifera* cutaneous infections have been underreported due to its incorrect identification based on earlier methods. We report an additional case of *E. spinifera* phaeohyphomycosis, the first to be definitively identified by sequencing. In addition, we summarize the variable clinical, histopathologic, and morphologic features, as well as treatment responses described in previously reported cutaneous infections caused by *E. spinifera*.

Keywords Phaeohyphomycosis, *Exophiala spinifera*, itraconazole, chromoblastomycosis

Introduction

Over one hundred species of dematiaceous fungi have been reported to cause infections in humans [1]. These etiologic agents contain melanin in their cell walls and are widely distributed throughout the environment. Members of the order Chaetothyriales are of particular significance as they are recurrent agents of infection. These fungi are oligotrophic, particularly occurring at low frequency in low-nutrient or hydrocarbon-polluted

environments [2]. Black moulds are etiologic agents of both chromoblastomycosis and phaeohyphomycosis. Chromoblastomycosis occurs most commonly in tropical environments and is a slow, progressive disease, often developing over several years. It is characterized clinically by verrucous lesions, usually on the lower extremities, and appears histopathologically as sclerotic bodies in tissue. Phaeohyphomycosis occurs worldwide, usually develops over a shorter duration, and presents in variable forms in tissue, including phaeoid yeastlike cells, hyphal elements, swollen cells, pseudohyphal elements, and/or moniliform hyphae.

The clinical syndromes of phaeohyphomycosis include allergic conditions, such as fungal allergic sinusitis [3,4], cutaneous disease (superficial, dermal or subcutaneous), as well as life-threatening disseminated

Received 20 January 2008; Final revision received 31 July 2008; Accepted 16 August 2008

Correspondence: Carrie Kovarik, 2 Maloney, 3600 Spruce Street, Philadelphia, PA 19104-4283, USA. Tel: +1 215 662 2737; fax: +1 215 615 0047; E-mail: carrie.kovarik@uphs.upenn.edu

infection [5], often characterized by brain abscess formation [6]. Acquisition of the organism varies among syndromes. Cutaneous disease presumably occurs through traumatic implantation of colonized material, while allergic sinusitis and disseminated infections including central nervous system involvement are likely acquired through inhalation of fungal structures.

Until recently, species identification of various genera has been based upon recognition of macroscopic and microscopic features of the fungus in culture, temperature studies, and a limited number of physiologic assays. These methods, however, have proved unreliable in differentiating very similar species, particularly in the genus *Exophiala* [7]. Definitive molecular techniques employing ribosomal DNA sequencing are now available for species identification [2]. This will become increasingly important, as treatment modalities have variable effectiveness across species [8]. *E. spinifera* has previously been implicated, primarily based on its morphology, in eleven cases of phaeohyphomycosis and three of chromoblastomycosis in the English literature. We report an additional case of phaeohyphomycosis caused by *E. spinifera* and review the literature to summarize patterns in clinical presentation, histologic appearance, morphologic features, molecular characterization, genetic variation, and treatment strategies.

Case report

A 49-year-old Caucasian man presented to the outpatient dermatology clinic of the University of Pennsylvania School of Medicine for evaluation of a non-healing ulcer on his right shin that had enlarged over the previous four months. Eleven years prior to presentation, he underwent a bilateral lung transplant for α_1 -antitrypsin deficiency, and his transplant was maintained with prednisone (10 mg/day), tacrolimus (2.5 mg twice/day) and mycophenolate mofetil (500 mg twice/day). He denied any systemic signs or symptoms, including other skin lesions, difficulty breathing, headache, or neurologic symptoms. The patient is a building contractor who works outdoors doing physical labor.

Physical exam revealed a cushingoid man with an edematous right lower leg and a large ulcer with an indurated border on the pretibial aspect. Two erythematous nodules were present in close proximity to the ulceration (Fig. 1). There was no palpable lymphadenopathy.

Two separate punch biopsies, one from the indurated ulcer border and another from a nearby nodule, each demonstrated pseudoepitheliomatous hyperplasia associated with a dense neutrophilic infiltrate. Pigmented

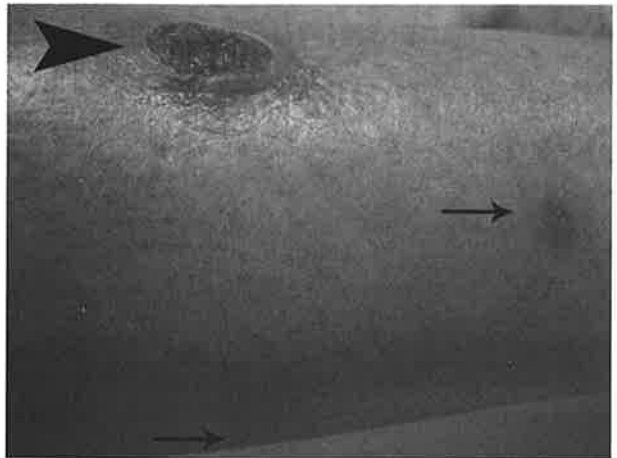


Fig. 1 Large ulcer (large arrow head) located on the right lower extremity with two smaller erythematous nodules (small arrows) in close proximity.

yeast-like and pseudohyphal elements were noted on routine hematoxylin and eosin staining and were highlighted with GMS, PAS and Fontana-Masson stains (Fig. 2).

A portion of the biopsied tissue and a separate wound swab were inoculated onto blood agar. Within one week, identical pigmented moulds were observed growing in cultures prepared from both specimens. The isolate was referred to the Fungus Testing Laboratory in the Department of Pathology at the University of Texas Health Science Center for further identification and accessioned into their stock collection as UTHSC 06-3355. Colonies were identified on potato flakes agar (PFA) [9] in ambient air with an on and off light cycle at 25°C. They measured 26 mm after 14 days and were moist initially but

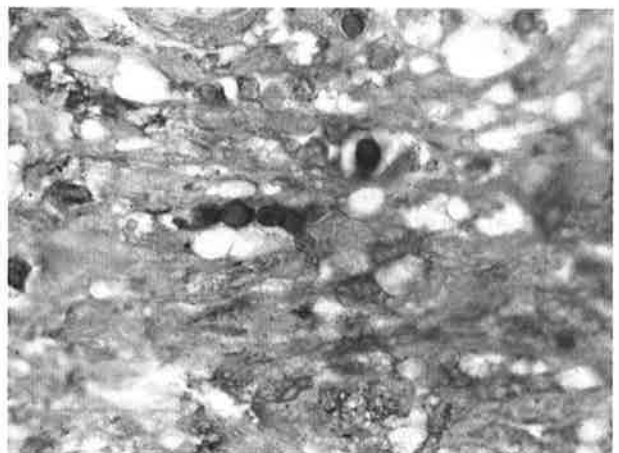


Fig. 2 GMS staining of a representative lesional skin biopsy highlights yeast-like fungal elements present both singly and forming pseudohyphae (GMS staining, 1000 \times).

became more filamentous at maturity (Fig. 3). Microscopic features included long, brown, septate, annellophores with obovoidal annelloconidia ($2\text{--}2.5 \times 3\text{--}4 \mu\text{m}$) produced at the apices of the conidiogenous cells as well as at intercalary loci (Fig. 4). A capsular black yeast synanamorph was also present. The isolate assimilated nitrate [10] and grew at 35°C but failed to grow at 40°C . Based upon the features noted, the isolate was identified as an *Exophiala* species, not *E. dermatitidis*.

The isolate was subsequently sequenced. After incubation overnight on potato dextrose agar at 30°C , a small amount of material was scraped off the plate and inoculated into $50 \mu\text{l}$ of Prepman Ultra reagent (Applied Biosystems, Inc., Foster City, CA). The cells were lysed according to the manufacturer's instructions and the suspension pelleted in a microfuge at $14,000 g$ for 10 min. PCR was performed on $5 \mu\text{l}$ of the supernatant in a 0.5 ml microfuge tube in a $50 \mu\text{l}$ reaction volume using Triple Master Taq DNA polymerase (Eppendorf/Brinkmann Instruments, Inc., Westbury, NY) according to the manufacturer's instructions. Two primer pairs were used to amplify two regions of the ribosomal repeat from the genomic DNA template. The ITS region (ITS1-5.8S-ITS2) was amplified using ITS1 ($5'\text{-TCCGTAGGTGAACCTGCGG-3'}$) and ITS4 ($5'\text{-TCCTCCGCTTATTGATATGC-3'}$) as described [11]. The D1/D2 region of the large ribosomal DNA subunit was amplified with primers NL-1 ($5'\text{-GCATATCAA-$

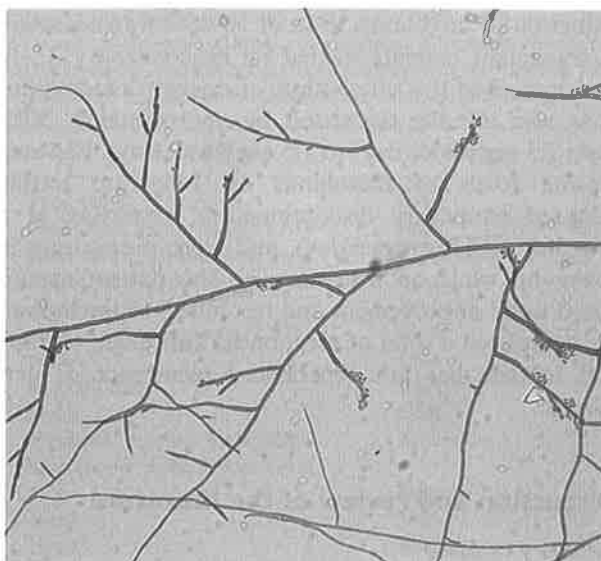


Fig. 4 Microscopic morphology of *Exophiala spinifera* showing long annellophores and annelloconidia, approximately $460 \times$.



Fig. 3 Colonial morphology of *Exophiala spinifera* after 14-day incubation at 25°C on potato flakes agar. Note moist area (arrow) of the yeast synanamorph.

$\text{TAAGCGGAGGAAAAG-3'}$) and NL-4 ($5'\text{-GGTCCGTGTTTCAAGACGG-3'}$) as described previously [12]. All PCR reactions were performed in a PTC-100 thermocycler (MJ Research, Watertown, MA) using the preprogrammed three-step protocol as the standard program for all reactions. PCR reactions were electrophoresed through a 0.7% agarose gel to confirm amplification. The remaining template DNA was then cleaned using a Qiaquick PCR purification kit (Qiagen, Inc., Valencia, CA). Sequencing was performed on both strands at the UTHSCSA Advanced Nucleic Acids Core Facility using all four PCR primers. Both ITS and D1/D2 sequences were used to perform nucleotide-nucleotide searches using the BLASTn algorithm at the NCBI website (<http://www.ncbi.nlm.nih.gov/BLAST/>). Identifications were made when BLAST searches yielded $>98\%$ identity. A definitive identification of *Exophiala spinifera* was made based upon a 100% and 99% identity with D1/D2 and ITS sequences, respectively, when compared with GenBank nucleotide deposits for this species and the Centraalbureau voor Schimmelcultures (CBS) *Exophiala* database. Both the ITS and D1/D2 sequences were submitted to Genbank under accession numbers EU257701 (ITS), and EU257702 (D1/D2).

The patient received oral itraconazole at an initial dose of 200 mg twice/day that was later tapered to 100 mg twice/day. Importantly, itraconazole inhibits the cytochrome P450 enzyme CYP3A4 and thus typically results in increased plasma levels of tacrolimus, part of the immunosuppressive regimen used with our patient. Previous reports have shown that a

reduction in tacrolimus dose of 50–66% was necessary in transplant patients started on itraconazole [13]. In anticipation of this interaction, our patient's tacrolimus dose was initially decreased by approximately 50%, from 2.5 mg (twice/day) to 1.5 mg (twice/day). Elevated plasma levels of tacrolimus on follow-up testing required temporary discontinuation, restarting at a low dose (0.5 mg twice/day), and close monitoring in follow-up while on itraconazole. The patient experienced rapid improvement and resolution of the lesions. He completed a total of six months antifungal therapy and to date has not experienced recurrence or new lesions.

Discussion and review of the literature

Summary of cases

Until recently, species identification of dematiaceous fungi has been based on appearance in tissue and in culture. By these criteria, *Exophiala spinifera* has been implicated in a total of 18 cases of clinical infections from ten different countries, with 14 cases appearing in the English literature. The initial case was reported in 1954 in a boy from India who succumbed to the infection. The pathogen was misclassified as *Hormodendrum dermatitidis* but was later reclassified based on modern morphologic criteria [14].

Of the 14 reported cases of *E. spinifera* infection in the English literature, 11 had histologic characteristics of phaeohyphomycosis and three of chromoblastomycosis (Table 1). Six cases were reported in children 13 years of age or younger – all had disseminated infection and two resulted in death. While both deaths occurred in healthy children, their clinical courses were marked by delayed diagnosis and ineffective treatment for a prolonged period prior to appropriate therapy. The remaining nine cases (including our report) were localized infections in adults and were easily treated in all but one. Clinical presentations were variable, including erythematous papules, verrucous plaques, deep subcutaneous abscesses, and an ulcer in our case. The site of initial infection (by history or exam) was on the face in four children but on an extremity in seven of nine adults. Various distant sites including skin, lymph nodes, bone, lung, and possibly the eye have been reported in cases involving dissemination. Unlike disseminated phaeohyphomycosis caused by other species [6], none involved the central nervous system.

Five of the nine adults were immunosuppressed, and all suppressive regimens included prednisone or prednisolone in doses from 2.5–16 mg/day. One adult

patient who was not on an immunosuppressive regimen during infection had a remote history of oral corticosteroids for asthma and her disease was exacerbated during pregnancy, a physiologically immunosuppressed state [14–27]. Our case is the 15th *E. spinifera* clinical infection reported in the English literature, and the first in which identification was definitively established by molecular characterization.

Histology

The histologic features of *E. spinifera* cutaneous infection are typical for the cutaneous manifestations of a deep fungal infection, including epidermal hyperkeratosis, acanthosis, pseudoepitheliomatous hyperplasia, and intraepidermal pustule formation. Dermal findings have included a dense mixed infiltrate, as well as suppurative and granulomatous infiltrates. Multinucleated giant cells are a common feature. Pigmented fungal elements can be detected most often in areas of inflammation, within or adjacent to multinucleated giant cells. GMS, PAS, and Fontana stains can be employed to highlight the fungal elements in instances involving lightly pigmented organisms. The fungal elements display one of two distinct histologic morphologies: a phaeohyphomycotic form or a chromoblastomycotic form. Three of the fourteen previously reported cases were classified as chromoblastomycosis and the remaining cases, including ours, as phaeohyphomycosis.

Specifically, phaeohyphomycosis is defined by the presence of dematiaceous fungi in tissue with a yeast-like, hyphal, or pseudohyphal morphology. These morphologies can be present uniformly or mixed. In culture-proven *E. spinifera* phaeohyphomycosis, the fungal elements have been described either as solitary forms or clusters of budding cells, thick-walled cells, or branched or septated hyphae. Other histologic descriptions have included budding yeast-like cells, thick walled chlamydo-spores, branched hyphae that were constricted at prominent septations, toruloid hyphal elements, and hyphae without prominent swellings. Some have observed hyaline to pale brown yeast cells with variable budding and the formation of pseudohyphae. In a case where the phaeohyphomycosis infection spread to an axillary lymph node, epithelioid cell granulomas with giant cells containing pigmented fungal elements were seen [14,15,20].

Chromoblastomycosis is defined by the presence of pigmented fungal elements in the form of muriform cells, thought to be an intermediate fungal form in the transition between the yeast and hyphal morphology. Other common terms for these elements include sclerotic bodies, fumagoid cells, copper pennies, Medlar

Table 1 Summary of cases of cutaneous *Exophiala spinifera* infection reported in the English literature. The first six cases listed are children, followed by six adult phaeohyphomycosis patients, followed by three adult chromoblastomycosis patients. *Abbreviations:* P, phaeohyphomycosis; C, chromoblastomycosis; pred, prednisone; prednisol, prednisolone; aza, azathioprine; tac, tacrolimus; myco mof, mycophenylate mofetil; LAD, lymphadenopathy. *Patient died. †Patient had a remote history of oral corticosteroids and disease was exacerbated during pregnancy.

Year/age/sex	Phaeo/Chromo	Immunosuppression	Initial Site	Additional sites	Country	Ref
1954/7/M*	P	N	L cheek	Face, trunk, extremities	India	13
2000/12/F	P	N	L forehead	Face, chest, arms, thighs; LAD	India	13
1980/6/M	P	N	R cheek	Face, earlobe, trunk, arm, lung	El Salvador	14
1994/12/M*	P	N	R face	Finger, elbow, toe; bone, regional LAD	Brazil	15
1993/13/M	P	N	unknown	'disseminated'	Pakistan	16
2008/10/M	P	N	legs	Face, arms, upper back; LAD	India	17
1968/72/F	P	N	Nose	None	USA	18
1983/60/M	P	Y-pred	R forearm	R hand, thigh	USA	19
1988/62/F	P	Y-gold + pred	L 4th finger	None	USA	20
1990/32/F	P	N†	R forehead	Forehead, inguinal folds, elbow; knee (bone), eyes, dissem LAD	Argentina	21
2005/85/F	P	Y-prednisol + aza	L arm	None	Japan	22
2008/49/M	P	Y-pred, tac, myco mof	R leg	None	USA	this case
1992/49/M	C	N	dorsal R 4th finger	None	Mexico	23
1993/62/M	C	Y-aza + pred	R 2nd finger	R forearm, elbow	USA	24
2002/78/M	C	N	L forearm	None	UK/Pakistan	25

bodies, and chlamydospores. In cases of *E. spinifera*, these elements have been described as round, chestnut brown or brownish in color, having a central pale area, and thick walls. Padhye *et al.* described the muriform cells as round-to-oval and ranging from 5 to 12 micrometers in diameter. They also described the presence of dematiaceous sporangium-like cells, which measured 12–20 micrometers in diameter and contained endocoonidia. Propagation of the muriform cells in tissue was demonstrated by planate division [24–26].

Cases of *E. spinifera* cutaneous infection with concurrent chromoblastomycosis and phaeohyphomycosis forms have not been reported to date. It is likely, therefore, that these diagnoses represent two poles of a spectrum of disease and that the development of either form in a particular patient may depend on a specific strain-individual-environmental interaction [24].

Mycology

The black yeast genus *Exophiala* contains several species capable of causing human disease but the identification and differentiation of these species remains problematic for routine clinical laboratories. Factors contributing to this difficulty include the pleomorphic nature of the genus, i.e., the ability of several species to form yeast and filamentous phases [28], limited physiologic differences among them, and their very similar macroscopic and microscopic morphologies. Prior to the development of molecular techniques to characterize these agents, the most common species reported in clinical cases were *E.*

jeanselmei and *E. dermatitidis*. Our understanding of the genus greatly expanded with the advent of sequencing methods and the analysis of sequence data of the ribosomal DNA (rDNA) Internal Transcribed Spacer (ITS) regions. This information permitted the differentiation of the genus into specific 'clades' or clusters of taxa, including the *E. spinifera* clade [8,29,30]. The common clinical species *Exophiala jeanselmei*, long known to be a heterogeneous collection of yeast-like fungi, was also further defined and shown to consist of several species including *E. heteromorpha*, *E. lecanii-corni*, *E. oligosperma* and *E. xenobiotica* [31], as well as *E. jeanselmei* in the restricted sense. In a recent study of the distribution of *Exophiala* species recovered from clinical specimens in the United States using ITS sequence data, the order of frequency for the most common isolates was *E. dermatitidis* (29.3%), *E. xenobiotica* (19.7%), *E. oligosperma* (18.6%), *E. lecanii-corni* (6.9%), and *E. phaeomuriformis* (6.4%). The recovery of *E. jeanselmei* and *E. spinifera* were at 3.7% and 2.7%, respectively [7]. Although *E. spinifera* is infrequently recovered and/or reported from clinical cases as a result of misidentification or lack of species identification, it is a cause of significant cutaneous disease.

Treatment

Definitive identification of the causative agent of phaeohyphomycosis is clinically important, as general species may vary in their susceptibility to antimicrobial agents [8]. *In vitro* studies suggest that *E. spinifera* is

most highly susceptible to itraconazole but poorly responsive to amphotericin B [8,32]. In a recent study evaluating the *in vitro* susceptibility patterns of clinically relevant *Exophiala* species in the US to the currently available antifungal agents, no significant differences were noted between species, except for amphotericin B resistance noted with three *E. attenuata* isolates [7]. A summary of the *in vitro* susceptibilities of *E. spinifera* to various drugs is listed in Table 2. One study showed that the addition of quinolones to itraconazole or amphotericin B improved their antimicrobial activity against *E. spinifera*; however this has not yet been tested clinically [33]. Importantly, it has been noted that *in vitro* susceptibility data seem to correlate poorly with *in vivo* efficacy [32].

Multiple antifungal therapeutic protocols have been utilized to treat infection with *E. spinifera*, including amphotericin B, ketoconazole, fluconazole, itraconazole, voriconazole, 5-fluorocytosine, terbinafine, griseofulvin, posaconazole, streptomycin, as well as physical treatment methods such as heat, cryosurgery and excision [8,14–26]. However, as detailed below, few of these treatments were ultimately found to be effective. Seven of the 12 patients who recovered from their infections, including our patient, were receiving itraconazole as part of their treatment regimens, and five were taking this medication as monotherapy. Four patients received amphotericin B in their initial treatment regimens of whom two required additional antifungal agents to obtain adequate clinical response, one died, and the last showed early improvement in short-term follow-up study [15–17,22]. In two cases there was increased resistance to itraconazole over the course of treatment that was verified by *in vitro* testing [15,25], and one report noted decreasing efficacy of multiple antifungal treatments over a prolonged course of treatment [22]. These investigations suggest that *E.*

spinifera possesses the ability to develop resistance to antifungal therapy. A recent report described that a panel of treatments that included itraconazole, terbinafine, fluconazole, and cryosurgery was ineffective in treating a case of *E. spinifera* infection [18]. Other treatments that were used in the remaining patients who recovered without itraconazole treatment included posaconazole, ketoconazole, and 5-fluorocytosine antifungal therapies, liquid nitrogen cryotherapy, and surgical excision without additional therapy.

Conclusions

In conclusion, phaeohyphomycosis caused by *E. spinifera*: (1) typically presents in children as a lesion on the face, usually from an unidentified exposure, whereas in adults it primarily occurs on an extremity and is secondary to traumatic implantation; (2) may develop into disseminated disease, which is more common in children than in adults and has a higher rate of mortality; (3) usually occurs in healthy children but more than half of reported adult patients were on an immunosuppressive regimen which included daily prednisone; (4) may present histologically with either a phaeohyphomycosis or chromoblastomycosis morphology; and (5) has been most successfully eradicated when treatment protocols included itraconazole.

Acknowledgements

The authors thank Elizabeth Thompson and Anna M. Romanelli for the morphologic identification and sequencing of the isolate, respectively.

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

References

- Revankar SG. Phaeohyphomycosis. *Infect Dis Clin North Am* 2006; **20**: 609–620.
- Prenafeta-Boldú FX, Summerbell RC, de Hoog GS. Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard. *FEMS Microbiol Rev* 2006; **30**: 109–130.
- Ferguson BJ. Definitions of fungal rhinosinusitis. *Otolaryngol Clin N Am* 2000; **33**: 227–235.
- Schubert MS. Allergic fungal sinusitis: pathogenesis and management strategies. *Drugs* 2004; **64**: 363–374.
- de Hoog GS, Guarro J, Gené J, Figueras MJ (eds), *Atlas of Clinical Fungi*, 2nd ed. Utrecht, The Netherlands and Universitat Rovira i Virgili Reus, Spain: Centraalbureau voor Schimmelfcultures, 2000.

Table 2 Minimal inhibitory concentrations in µg/ml of drugs tested against *Exophiala spinifera*. MIC listed is the geometric mean of multiple strains tested in each reference. *Abbreviations:* ITZ, itraconazole; VCZ, voriconazole; FCZ, fluconazole; TBF, terbinafine; 5-FC, 5-fluorocytosine; AMB, amphotericin B; POS, posaconazole; nd, not done. *This reference determined MIC₅₀ and MIC₉₀. The MIC listed is for MIC₅₀, consistent with the other references. † This reference tested susceptibility of environmental and clinical strains at various temperatures. The MIC listed is for clinical isolates grown at 35°C, consistent with the other references.

Ref	ITZ	VCZ	FCZ	TBF	5-FC	AMB	POS
7*	0.03	0.125	nd	nd	nd	0.5	<0.015
8	0.05	0.3	55.05	0.26	3.44	0.7	nd
32†	0.06	0.27	45.25	0.3	5.66	0.92	nd

- 6 Revankar SG, Sutton DA, Rinaldi MG. Primary central nervous system phaeohyphomycosis: a review of 101 cases. *Clin Infect Dis* 2004; **38**: 206–216.
- 7 Zeng JS, Sutton DA, Fothergill AW, et al. Spectrum of clinically relevant *Exophiala* species in the U.S.A. *J Clin Microbiol* 2007; **45**: 3713–3720.
- 8 Vitale RG, de Hoog GS. Molecular diversity, new species and antifungal susceptibilities in the *Exophiala spinifera* clade. *Med Mycol* 2002; **40**: 545–556.
- 9 Rinaldi MG. Use of potato flakes agar in clinical mycology. *J Clin Microbiol* 1982; **15**: 1159–1160.
- 10 Pincus DH, Salkin IF, Hurd NJ, Levy IL, Kemna MA. Modification of potassium nitrate assimilation test for identification of clinically important yeasts. *J Clin Microbiol* 1988; **26**: 366–368.
- 11 White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR-Protocols and Applications – A Laboratory Manual*. New York: Academic Press, 1990: 315–322.
- 12 Kurtzman CP, Robnett CJ. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal DNA gene. *J Clin Microbiol* 1997; **35**: 1216–1223.
- 13 Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. *Pharmacotherapy* 2006; **26**: 1730–1744.
- 14 Rajendran C, Khaitan BK, Mittal R, et al. Phaeohyphomycosis caused by *Exophiala spinifera* in India. *Med Mycol* 2003; **41**: 437–441.
- 15 Padhye AA, Ajello L, Chandler FW, et al. Phaeohyphomycosis in El Salvador caused by *Exophiala spinifera*. *Am J Trop Med Hyg* 1983; **32**: 799–803.
- 16 Campos-Takaki GM, Jardim ML. Report of chronic subcutaneous abscesses caused by *Exophiala spinifera*. *Mycopathologia* 1994; **127**: 73–76.
- 17 Mirza SH, Hannan A, Ahmad A, Ahmad M. Subcutaneous phaeohyphomycosis. *J Infect* 1993; **27**: 75–78.
- 18 Singal A, Pandhi D, Bhattacharya SN, et al. Phaeohyphomycosis caused by *Exophiala spinifera*: a rare occurrence. *Int J Dermatol* 2008; **47**: 44–47.
- 19 Nielsen HS, Conant NF. A new human pathogenic *Phialophora*. *Saboraudia* 1967–1968; **6**: 228–231.
- 20 Padhye AA, Kaplan W, Neuman MA, Case P, Radcliffe GN. Subcutaneous phaeohyphomycosis caused by *Exophiala spinifera*. *Saboraudia* 1984; **22**: 493–500.
- 21 Kotylo PK, Israel KS, Cohen JS, Bartlett MS. Subcutaneous phaeohyphomycosis of the finger caused by *Exophiala spinifera*. *Am J Clin Path* 1989; **91**: 624–627.
- 22 Negroni R, Helou SH, Petri N, et al. Case study: posaconazole treatment of disseminated phaeohyphomycosis due to *Exophiala spinifera*. *Clin Infect Dis* 2004; **38**: e15–e20.
- 23 Takahara M, Imafuku S, Matsuda T, et al. Concurrent double infections of the skin: Phaeohyphomycosis and nocardiosis in a patient with idiopathic thrombocytopenic purpura. *J Am Acad Dermatol* 2005; **53**: S277–S280.
- 24 Barba-Gomez JF, Mayorga J, McGinnis MR, et al. Chromoblastomycosis caused by *Exophiala spinifera*. *J Am Acad Dermatol* 1992; **26**: 367–370.
- 25 Padhye AA, Hampton AA, Hampton MT, et al. Chromoblastomycosis caused by *Exophiala spinifera*. *Clin Infect Dis* 1996; **22**: 331–335.
- 26 Tomson N, Abdullah A, Maheshwari MB. Chromomycosis caused by *Exophiala spinifera*. *Clin Exp Dermatol* 2006; **31**: 239–241.
- 27 Hunt JS. Stranger in a strange land. *Immunol Rev* 2006; **213**: 36–47.
- 28 deHoog GS, Takeo K, Yoshida S, et al. Pleoanamorphic life cycle of *Exophiala (Wangiella) dermatitidis*. *Antonie van Leeuwenhoek* 1994; **65**: 143–153.
- 29 deHoog GS, Poonwan N, Gerrits van den Ende AHG. Taxonomy of *Exophiala spinifera* and its relationship to *E. jeanselmei*. *Stud Mycol* 1999; **43**: 133–142.
- 30 deHoog GS, Vicente V, Caligiorno RB, et al. Species diversity and polymorphism in the *Exophiala spinifera* clade containing opportunistic black yeast-like fungi. *J Clin Microbiol* 2003; **41**: 4767–4778.
- 31 de Hoog GS, Zeng JS, Harrak MJ, Sutton DA. *Exophiala xenobiotica* sp. nov., an opportunistic black yeast inhabiting environments rich in hydrocarbons. *Antonie van Leeuwenhoek* 2007; **90**: 257–268.
- 32 Vitale RG, de Hoog GS, Verweij PE. *In vitro* activity of amphotericin B, itraconazole, terbinafine, and 5-fluocytosine against *Exophiala spinifera* and evaluation of post-antifungal effects. *Med Mycol* 2003; **41**: 301–307.
- 33 Vitale RG, Afeltra J, de Hoog GS, Rijs AJ, Verweij PE. *In vitro* activity of amphotericin B and itraconazole in combination with flucytosine, sulfadiazine and quinolones against *Exophiala spinifera*. *J Antimicrob Chemother* 2003; **51**: 1297–1300.

This paper was first published online on iFirst on 19 December 2008.