

Aspergillus terreus complex: an emergent opportunistic agent of Onychomycosis

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Summary

The incidence of onychomycosis due to non-dermatophyte moulds (NDM) is increasing. *Aspergillus terreus* is relatively undocumented as an agent of this fungal infection. The aim of this work is to show the prevalence of onychomycosis caused by *A. terreus* and to describe its clinical features. Nail samples were collected for microscopic examination and culturing in selective media. All cases of onychomycosis due to NDM were confirmed by a second sample. *Aspergillus terreus* isolates were identified through their morphological characteristics and using molecular methods. A total of 2485 samples were obtained. Positive cultures were obtained in 1639 samples. From 124 NDM confirmed cultures, 23 were identified as *A. terreus* (18.5%). Superficial white onychomycosis was the most frequent clinical pattern. A high percentage was found in fingernails. The prevalence of *A. terreus* in this study considerably exceeded the percentages reported by other authors. Onychomycosis due to *A. terreus* presents similar clinical patterns to those caused by dermatophytes, but is difficult to eradicate and is associated with less predictable treatment outcomes. Better knowledge of the aetiology of *A. terreus* may be important for accomplishing more accurate and effective treatment.

Key words: *Aspergillus terreus* complex, non-dermatophytes onychomycosis, clinical types of onychomycosis.

Introduction

Onychomycosis is defined as a fungal infection of the nail plate and surrounding tissues and is considered a major health disorder and a growing public health problem.^{1–4} Fungal infections represent between 40% and 60% of nail diseases, and their prevalence in the general population has been estimated between 1.7% and 13%.^{1,5–7} The frequency of these infections is low during childhood and increases considerably in people older than 60 years of both genders.^{1,5–11}

Agents causing onychomycosis can be divided into three groups: dermatophytes, yeast and non-dermatophyte moulds (NDM). The last is not a homogeneous group and includes species belonging to several and different families and genera. The literature about the pathogenic role of NDM is controversial because they can act as contaminants, colonisers or pathogens,^{6,7,10,12,13} even though the incidence of onychomycosis due to NDM is increasing. Several reasons have been suggested for this increase, including the following: an increase in cultural changes towards generalised sports practice, massive use of public swimming pools and bathrooms, manicures, occlusive footwear, populations at risk such as immunocompromised patients, patients with diabetes and peripheral vascular disease and, finally, the awareness concerning the need to know the aetiology of these mycoses to apply an effective treatment can be mentioned.^{5,10,14} It is important to know the causative

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agent of a NDM onychomycosis, especially in older people and in immunosuppressed patients. A sub-diagnosis in these populations can lead to serious complications as these infections are aggressive and might open the door to systemic infections.^{14,15}

There is a long list of moulds occasionally isolated from infected nails, but the most commonly mentioned are *Scopulariopsis brevicaulis*, *Aspergillus* species, *Fusarium* species, *Acremonium* species and *Scytalidium* species.^{5,6,12,13} In Argentina, the most frequently isolated are *Fusarium* species, *Acremonium* species, *Scopulariopsis* species and *Aspergillus* species.^{8,16,17}

The first cases of primary nail invasion by *Aspergillus* were recorded by Bereston and Kiel and by Moore and Weiss in 1940.¹² This genus is regarded as an emerging fungal agent of toenail onychomycosis, even though few species have been determined as such.¹² *Aspergillus terreus* complex (*A. terreus* complex), *A. flavus*, *A. versicolor* and *A. sydowii* have been reported to cause this infection.^{6,12}

Aspergillus terreus complex is a worldwide hyaline mould widely found in soil and air.¹⁸ As an opportunistic agent, it causes both systemic and superficial infections; among the latter, the most frequently reported is toenail onychomycosis, usually affecting the first toes.¹²

As we have detected several cases of onychomycosis caused by *A. terreus* complex, the aim of this study is to show the prevalence of onychomycosis caused by this agent and to describe its clinical features.

Materials and methods

In the city of Resistencia, located in north-eastern Argentina (27°27'05"S, 58°59'12"W), patients with fingernail and toenail lesions were studied between January 2007 and February 2011. A personal record was performed with the patient's data (age, gender and underlying pathology) and data on the lesion, including localisation and clinical patterns, distal/lateral subungual onychomycosis (DLSO), superficial white onychomycosis (SWO), onycholysis and paronychia.

The patients were prepared as follows: (i) All systemic antifungal treatment was ceased 15–20 days before taking the clinical sample. If topical treatment was used, this was suspended 72 h before sampling. (ii) Nail brushing was performed with water and soap twice a day, 3 days before sampling, avoiding the use of talcum powder, creams or nail varnish. (iii) Patients attended the laboratory in occlusive footwear with socks to avoid environmental contamination.^{5,16}

Samples were obtained from the infected nail by clipping with a nail clipper or scraping with a sterile scalpel and were then stored in Petri dishes until processing.

A direct microscopic examination of the nail scraping with 40% potassium hydroxide was performed under 40× magnification.¹⁶ The specimens were cultured in duplicate in potato dextrose agar (PDA) with antibiotics (chloramphenicol 250 mg l⁻¹) and in PDA with cycloheximide. These four tubes were incubated for at least 2 weeks at 28 °C.

In all cases where direct microscopic examination was positive for hyaline hyphae and growth of the NDM was observed in the absence of dermatophyte growth, a second sample was taken to confirm the aetiology of the lesion.

When an *Aspergillus* species strain was isolated, it was sub-cultured on Czapek yeast extract agar (25 °C) and on malt extract agar (25 °C); both culture media are specific for *Aspergillus* identification. Species typification was performed according to general keys for species of this genus by studying the macro and micro-morphological characteristics of the colonies.^{19–22} *Aspergillus terreus* complex strains were sent for their molecular identification to the Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria.

This study was approved by our Institutional Review Board.

Results

A total of 2485 samples were obtained from 2178 patients (fingernails: 679; toenails: 1806). The difference between patients and samples occurred as some patients came twice and others had lesions in both fingernails and toenails.

From 2485 samples, positive cultures were obtained in 1639 samples; 807 (49.24%) were dermatophytes, 618 (37.70%) were yeast, 167 (10.20%) were NDM and 47 (2.86%) were dermatophytes associated with yeast. Of the 167 NDM positive cultures, 124 (74%) were confirmed with a second sampling. The prevalence of NDM onychomycosis among 1639 positive cultures was 7.56% (124/1639). From 124 NDM confirmed cultures, 29 belonged to the *Aspergillus* genus, of which 23 were identified as *A. terreus*. Therefore, the prevalence of onychomycosis by *A. terreus* among the 124 NDM positive cultures was 18.5% (23/124).

Throughout the study, an increase of the cases of onychomycosis by *A. terreus* was observed, with four cases in the first 2 years (2007–2008) and 19 cases

in the last 2 years (2009–2010). The age of the 23 patients with onychomycosis by *A. terreus* was between 27 and 72 years (average age 50.3 years). Twenty-two patients were women. None of them presented an underlying pathology. Six cases of *A. terreus* were in the fingernail (five women and one man) and 17 in the toenail (all women).

A significant difference ($P < 0.05$) in age among patients with fingernail lesions and those with toenail lesions was observed. The average age among the former was 60.17 years and the average age among the latter was 46.76 years.

The observed clinical patterns in fingernail were DLSO (33.33%), SWO (33.33%) and Onycholysis (33.33%) and in toenail SWO (52.94%) was the most frequent clinical pattern followed by DLSO (41.98%) and DLSO + SWO (5.88%). Pictures of SWO and onycholysis are shown in Figs 1 and 2.

The sensitivity of direct examination in cases of onychomycosis due to *A. terreus* was 100%. All direct microscopic examinations showed wide, septate, hyaline hyphae with dichotomous branching and, in some cases, even conidiophores with the typical radiating conidial heads were observed (Fig. 3).

Discussion

The minimum criteria accepted for the diagnosis of onychomycosis due to NDM are as follows: (i) finding of hyphae with or without spores by direct microscopic examination of the clinical sample, (ii) isolation of NDM in the absence of dermatophytes and (iii) isolation of the same fungal species of NDM in a second sample from the same nail.^{1,5,8,23–25} The requirements



Figure 2 Fingernail with onycholysis.

for establishing the diagnosis of NDM onychomycosis are difficult to fulfil in daily practice as only a few patients return for the second sampling. In this study, 74% of NDM positive cultures were confirmed with a second sample.

The findings of our study, both in terms of the average age of patients with onychomycosis due to *A. terreus* and the high percentage of toenail lesions, are consistent with numerous reports about onychomycosis by NDM.^{8,10,12–14,23} Few studies on onychomycosis due to NDM have been reported for *A. terreus* complex



Figure 1 Toenail with superficial white onychomycosis.

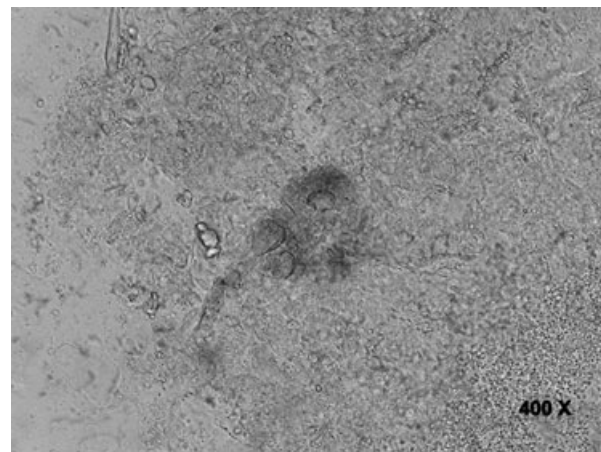


Figure 3 Direct microscopic exam showing conidiophores with the typical radiating conidial heads.

or identified isolates of *Aspergillus*.^{6,8,10,14,16,26} We have only found reports of onychomycosis by *A. terreus* complex in Siena (Italy) 5.6%,¹² in Mexico City (Mexico) 10.2%,¹³ in London (England) 2.5%² and in Copenhagen (Denmark), where four cases were reported.²⁷

The prevalence of *A. terreus* found in our study exceeds the percentages mentioned above. The demand for onychomycosis studies did not change during the 4 years of the study, even though the presence of *A. terreus* increased by fivefold in this period. Inferring that the same standard was used in the mycology laboratory for the diagnosis of onychomycosis by NDM, these data would suggest geographical variation in the prevalence of the species.

Aspergillus terreus complex has been identified as an emergent species causing onychomycosis in older patients with peripheral circulatory disorders.⁸ None of our patients reported underlying health conditions. A significant difference ($P < 0.05$) in terms of gender was found in our patients with onychomycosis due to *A. terreus*. The female : male ratio was 22 : 1, which agrees with other publications about onychomycosis due to NDM.^{10,12–14}

According to the literature on this subject, onychomycosis due to *Aspergillus* species presents as DLSO and SWO with SWO in the toenail being the most common pattern in infections due to this complex.^{1,5,10,12,23,27,28} Our findings in toenails are in agreement with this, and in the fingernail lesions, the three clinical patterns were found in the same percentage. In addition, none of the consulted authors mentioned onycholysis by *A. terreus* complex either in fingernails or toenails.^{5,10,12,23,27,28} Although *A. terreus* complex is rarely reported in fingernails, we found a high percentage in this location.

Fingernail lesions were more common in older patients. This may be due to the rate of growth of fingernails is often decreased in the elderly which make them more susceptible to developing onychomycosis.^{1,5}

Aspergillus terreus complex is an alarming emergent opportunistic pathogen, which is considered one of the less sensitive species to available antifungal agents in clinical use. Onychomycosis caused by the *Aspergillus* genus can be hard to eradicate. Moreover, a nail infection due to *Aspergillus* species in immunosuppressed patients may disseminate and cause a systemic infection that can lead to death.¹⁵ The *in vivo* and *in vitro* activity of terbinafine antifungal agents has been widely demonstrated against *Aspergillus* species in these infections.^{12,25,29,30} Moreover, the *in vitro*

activity of terbinafine is more effective than itraconazole against different *Aspergillus* species. Thus, therapeutic choices are very scarce.^{12,29–31}

Onychomycosis caused by *A. terreus* complex presents similar clinical patterns to those caused by dermatophytes,^{25,32} with less predictable treatments and a high rate of therapeutic failure. Better knowledge of the aetiological agents and their *in vitro* sensitivity is important for accomplishing more accurate and effective treatments.^{32,33}

This study contributes to the scarce knowledge about the prevalence of this opportunistic agent.

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Conflict of interest

The authors have no conflict of interest to declare.

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