mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Original article

Non-dermatophyte mould onychomycosis: a clinical and epidemiological study at a dermatology referral centre in Bogota, Colombia

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Summary

Worldwide prevalence of non-dermatophyte mould onychomycosis has increased in recent years; however, available information on the topic is confusing and oftentimes contradictory, probably due to the small number of reported cases. The aim of this study was to determine and describe the aetiological agents, as well as the epidemiological and clinical characteristics of non-dermatophyte mould onychomycosis in a dermatology referral centre in Bogota, Colombia. A cross-sectional descriptive study was conducted between January 2001 and December 2011 among patients who attend the National Institute of Dermatology with a confirmed diagnosis of onychomycosis by non-dermatophytes moulds. There were 317 confirmed cases of non-dermatophyte mould onychomycosis in 196 women and 121 men whose average age was 43 years. Twenty-seven per cent of them had a history of systemic disease. The habit of walking and showering barefoot was the major infection-related factor. Distal and lateral subungual presentation was the most common pattern of clinical presentation. The most frequent non-dermatophyte mould was *Neoscytalidium dimidiatum* followed by *Fusarium* spp. No relationship was observed with predisposing factors previously reported in the literature. Clinical features found in this population are indistinguishable from onychomycosis caused by dermatophytes. High prevalence of *N. dimidiatum* found here was in contrast to a large number of studies where other types of moulds predominate.

Key words: Non-dermatophytes, moulds, onychomycosis, epidemiology, *Neoscytalidium dimidiatum*.

Introduction

The prevalence of non-dermatophyte mould infection varies from 1% to 68% of all onychomycosis, this depends on the diagnostic criteria used and where the study has been conducted.[1–5] The aetiological agents isolated may also vary, being *Acremonium* spp., *Scopulariopsis* spp. and *Aspergillus* spp. the most common moulds in the United States,[6] Europe[2,7–9] and Asia.[10,11]

The risk factors of suffering from onychomycosis can be divided into personal and environmental factors. The first group includes genetic predisposition, diabetes, lower extremity venous disease, microcirculatory disorders, Raynaud's phenomenon, collagen diseases, finger deformities, dystrophic nails, ageing, chronic skin diseases such as psoriasis, ichthyosis and atopic dermatitis, mucocutaneous candidiasis, tinea pedis, cancer, systemic corticosteroid therapy, HIV infection and other immune deficiencies.[12,13] The most important risk factors are tropical or subtropical

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Submitted for publication 23 July 2013
Revised 22 October 2013
Accepted for publication 26 October 2013

climate, occlusion and excessive sweating on the hands or feet, repeated trauma, detergents, abrasive liquids and food handling, wearing boots and closed shoes, contact with contaminated tools and equipment for cosmetic treatment of the hands and fingernails (manicure and pedicure), smoking, contact with animals such as family pets, use of potentially contaminated swimming pools and public showers.[14]

One of the major controversies in the diagnosis of onychomycosis is the identification of filamentous fungi or non-dermatophyte moulds, soil saprophytes and common phytopathogens.[3] Although moulds in this context have traditionally been viewed as contaminant agents and secondary pathogens (isolated from dystrophic or colonised nails) due to their virtual inability to invade keratinised tissues;[3,15,16] actually in fact, they are a major cause of skin lesions and onychomycosis which contribute to a progressive increase in the number of cases in different countries[9,17,18] after the first report in the early 1970s.[19,20]

The increase in the frequency of isolation of non-dermatophyte moulds may be explained by the following: increased life expectancy of susceptible populations such as the elderly and the immunesuppressed, social and cultural changes resulting from migration, more general sports practice, use of swimming pools and public showers, use of occlusive footwear, manicure and pedicure with non-sterile tools.[21] Scher *et al.* [22], in their study on onychomycosis in the geriatric population in the southern United States, documented an increase in nail infections by dermatophytes and non-dermatophytes moulds, suggesting that age, peripheral circulation, health status, environment and geography are related to the pathogenesis of onychomycosis. The modern facilities for international transportation, trade and tourism could also be related to the simultaneous increase in onychomycosis moulds in different parts of the world. In Colombia, violence and social conflict have led to the displacement of people from rural areas to the cities, with consequent poverty and overcrowding conditions.

The aim of this study was to determine and describe the aetiological agents and the epidemiological and clinical characteristics of non-dermatophyte onychomycosis mould in a dermatology referral centre in Bogota, Colombia.

Patients and methods

A cross-sectional retrospective study was conducted among patients with diagnosis of onychomycosis by non-dermatophytes moulds, who sought treatment at the outpatient clinic of the Centro Dermatologico Federico Lleras Acosta, the National Institute of Dermatology of Colombia, in the 11-year period between January 2001 and December 2011. Clinical and epidemiological characteristics were assessed in patients with laboratory-confirmed cases based on information obtained from the medical records and database of the Medical Mycology Laboratory.

All patients with a clinical suspicion of onychomycosis were referred to the Medical Mycology Laboratory for a complete mycological study. Clinical presentation of onychomycosis, skin abnormalities observed during physical examination (fungal infections such as ringworm of the foot) and changes in the nail appearance: lesions such as spots, discoloration (chromonychia), abnormal thickening (pachyonychia), subungual hyperkeratosis and nail separation (onycholysis) were registered in medical records. All subjects completed a questionnaire that included demographics on age, gender, place of origin and place of residence (urban or rural), occupation, history of systemic diseases (chronic lower extremity venous disease, diabetes, thyroid disease, HIV infection and any other immune suppression), history of trauma, evolution of the onychomycosis and predisposing factors (contact with animals such as family pets, walking barefoot, showering barefoot, use of swimming pools and public showers, manicure and pedicure and sports participation).

The nail collection technique for mycological examination varied depending on the clinical presentation of onychomycosis: in cases of distal and lateral subungual onychomycosis (DLSO) the underside of the nail plate and the hyponychium were scraped off from the distal part up to the more proximal part of the lesion. In proximal subungual onychomycosis (PSO) the nail was drilled at several points with the tip of a scalpel blade on the affected area to form a channel that would allow for subungual material to be obtained by scraping. In white superficial onychomycosis (WSO) the affected surface of the nail was scraped. For total dystrophic onychomycosis (TDO) the samples were collected by combining the techniques described above. The material was placed upon a microscope slide, and after being mixed, was inoculated in at least 10 points with Sabouraud dextrose agar with antibiotics. The microscopic preparation of the sample was mounted in 40% potassium hydroxide (KOH) and observed after 24 h. The cultures were incubated at 25 °C and examined every 4 days for 4 weeks. The identification of microorganisms was based on the observation and description of

the macroscopic and microscopic characteristics of the colonies obtained in the culture.

We identified patients with confirmed diagnosis of onychomycosis by non-dermatophytes moulds according to the criteria proposed by English which include: direct examination (KOH) positive with the presence of hyphae in the keratin, absence of a dermatophyte in culture and growth of the same mould in at least two consecutive cultures.[23,24]

Results

There were a total of 317 confirmed cases of non-dermatophyte mould onychomycosis in 196 women and 121 men with a range in age of between 9 and 85 years, and an average age of 43 years in both genders. The majority of patients (90%) came from Bogota. They were administrative assistants and professional or technical staff (50.4%), although there was also a substantial percentage of housewives (20.5%) and merchants (13.2%). The average disease duration was 59 months (range: 1–600). Fifty per cent of cases had less than 60 months of duration, with a median of 36 months.

The most important infection-related factor identified in this group was the direct contact with the soil, which includes the habit of walking and showering barefoot. Other findings cited in the literature as risk factors were identified in this population: clinical signs suggestive of tinea pedis, contact with animals (dogs, cats, ornamental birds and rabbits), repeated nail trauma, use of swimming pools and public showers, manicure or pedicure and sports participation (Table 1). Unexpectedly, *Neoscytalidium dimidiatum* was isolated as the causative agent of onychomycosis in 69% of patients who had clinical signs of tinea pedis (74/107).

Twenty-seven per cent of patients had a history of systemic and chronic diseases such as lower extremity venous disease (40/317), hypothyroidism (23/317), diabetes mellitus (8/317) and psoriasis (7/317). On the other hand, five patients had psoriatic (3/317) and lichen planus (2/317) nail changes that could promote infection with fungi. Finally, two patients were in treatment with immunosuppressive drugs, one had major depression and another had been diagnosed with HIV infection (without AIDS diagnosis).

The most common location of onychomycosis was bilateral great toenail (115/317). Seven patients had any other toenails affected and three had fingernail involvement alone. In these fingernails onychomycosis isolated moulds were *Penicillium* spp., *N. dimidiatum* and *Fusarium* spp.

We were unable to classify the clinical presentation in 54 cases (17%) because of the poor quality of some medical records. Nevertheless, DLSO was found in 240 patients (75.7%) and it was the most common clinical presentation in toenails. TDO was found in 16 patients (5%). WSO and PSO were presented in four and three patients, respectively, and these seven cases were caused by *N. dimidiatum* and *Acremonium* spp. (Table 2). Two of the three patients with fingernail lesions, caused by *N. dimidiatum* and *Penicillium* spp., had erythema and swelling of the periungual nail fold. In contrast, no patient with infected toenails presented those inflammatory changes.

Other nail changes were chromonychia (301/317), pachyonychia (250/317) and onycholysis (211/317). Changes in nail colour included yellow, white, brown and green discoloration, besides some colour combinations (Table 3).

The most frequently isolated five moulds were *N. dimidiatum* (in 178/56%), *Fusarium* spp. (in 100/31%),

Table 1 Factors associated with non-dermatophyte moulds onychomycosis.

Habits	*n*	%
Close contact with soil surface	173	54.6
Tinea pedis	107	33.8
Contact with animals such as family pets	72	22.7
Repeated nail trauma	50	15.8
Use of swimming pools and public showers	27	8.5
Manicure or pedicure	15	4.7
Sports participation	11	3.5

Table 2 Morphological pattern of onychomycosis and isolated moulds.

	Type of onychomycosis				
Mould	DLSO	SWO	PSO	TDO	Undefined
Neoscytalidium dimidiatum	141	3	2	7	25
Fusarium spp.	73	0	0	7	20
Acremonium spp.	10	1	1	1	5
Aspergillus spp.	8	0	0	0	2
Penicillium spp.	3	0	0	0	0
N. dimidiatum and *Acremonium* spp.	2	0	0	0	0
Paecilomyces spp.	1	0	0	0	0
Scedosporium spp.	1	0	0	0	0
Scopulariopsis brevicaulis	0	0	0	1	0

DLSO, distal and lateral subungual onychomycosis; SWO, superficial white onychomycosis; PSO, proximal subungual onychomycosis; TDO, total dystrophic onychomycosis.

Table 3 Nail discoloration.

Colour	*Neoscytalidium dimidiatum*	*Fusarium*	*Aspergillus*	*Acremonium*	Other moulds	Mixed infections	*n*	%
Yellow	79	33	5	7	5	2	131	41.3
Yellow-white	28	22	2	3	–	–	55	17.4
Yellow-brown	26	17	1	3	1	–	48	15.1
White	14	10	–	–	–	–	24	7.6
Brown	7	3	–	1	–	–	11	3.5
White-yellow-brown	7	1	–	–	–	–	8	2.5
White-brown	3	1	–	1	–	1	6	1.9
Green-yellow	2	3	–	–	–	–	5	1.6
Green	1	1	–	–	–	–	2	0.6
Yellow-brown-black	2	–	–	–	–	–	2	0.6
Brown-black	–	1	–	1	–	–	2	0.6
Another combination of colours	3	3	1	1	–	1	9	3.2
No discoloration	6	5	1	1	–	1	14	4.4
Total	178	100	10	18	6	5	317	100

Table 4 Isolated non-dermatophyte moulds and mixed infections.

Moulds	*n*	%
Neoscytalidium dimidiatum	178	56.2
Fusarium spp.	100	31.5
Acremonium spp.	18	5.7
Aspergillus spp.	10	3.2
Penicillium spp.	3	0.9
Paecilomyces spp.	1	0.3
Scedosporium spp.	1	0.3
Scopulariopsis spp.	1	0.3
N. dimidiatum and *Acremonium* spp.	2	0.6
N. dimidiatum and *Aspergillus* spp.	1	0.3
N. dimidiatum and *Fusarium* spp.	1	0.3
Acremonium spp. and *Fusarium* spp.	1	0.3
Total	317	100

Acremonium spp. (in 18/6%), *Aspergillus* spp. (10/3%) and *Penicillium* spp. (3/1%). Other isolated moulds were *Paecilomyces* spp., *Scedosporium* spp. and *Scopulariopsis* spp. with only one case. Interestingly, there were five cases of mixed infection, defined as the isolation of two different moulds in the same patient (Table 4).

Discussion

Dermatophytes are the main cause of onychomycosis worldwide.[25–27] However, the identification of different non-dermatophyte moulds as aetiological agents of onychomycosis and the increasing number of reported cases in the literature during the last 15 years has brought about great interest in this topic.

The real prevalence of non-dermatophyte moulds onychomycosis remains unknown. In a systematic review published in 2012, numerous inconsistencies were found and also uncertainties in the diagnostic criteria used in 21 published studies in the English language,[26] highlighting the limitations of the available information. Gupta *et al.* described the main points used for the diagnosis of non-dermatophyte moulds onychomycosis:

- Microscopic identification of fungal structures in KOH preparations of nail scrapings.
- Identification of the non-dermatophyte mould in culture.
- Growth of the same mould in culture from a second sample.
- Colony counts according to the inoculation number.
- No growth of any dermatophyte or yeast.
- Histopathological examination.

During the last 10 years, non-dermatophyte moulds have been the principal aetiological agents of onychomycosis in some countries in Asia and Africa, exceeding the number of dermatophyte infections (Table 5). In Colombia, their prevalence varies between 12% and 21%, similar to North American and European studies data.

Onychomycosis is more frequent in women,[25,28,29] and this could be related to their concern about nail changes, guiding them to seek early medical advice.[30,31] It has been suggested that wearing female open toe shoes favours trauma and direct contact with the ground surface, where saprophyte moulds live.[32] In the population of this study the male-to-female ratio was 1 : 1.6, similar to the results of Bonifaz *et al.* [31] in Mexico and Farwa *et al.* [1] in Pakistan, but

Table 5 Worldwide prevalence rates of onychomycosis caused by non-dermatophyte moulds.

Prevalence (%)	Year of publication	Cases (*n*)	Country	Most common causative agent	References
68	2011	32	Pakistan	*Alternaria alternata*	[1]
59	2006	19	Egypt	*Aspergillus niger*	[50]
51	2004	33	Thailand	*Neoscytalidium dimidiatum*	[70]
27	2007	49	India	*Aspergillus* spp.	[71]
26	2012	37	Costa Rica	*Fusarium* spp.	[79]
21	2005	928	Colombia	*Fusarium* spp.	[29]
21	2000	44	United States	*Fusarium* spp.	[80]
19	2009	41	Iran	*Aspergillus* spp.	[10]
15	2000	196	Spain	*Scopulariopsis brevicaulis*	[9]
14	2000	59	Italy	*Fusarium* spp.	[23]
14	2004	21	Colombia	*Fusarium* spp.	[30
13	2005	532	Italy	*S. brevicaulis*	[8]
12	2003	310	Colombia	*Fusarium* spp.	[21]
11	2010	47	Iran	*Aspergillus* spp.	[34]
9	2000	51	Italy	*S. brevicaulis*	[2]
9	2006	48	Greece	*S. brevicaulis*	[7]
9	2005	33	Turkey	*A. niger*	[11]
7	2009	13	Brazil	*Fusarium* spp.	[28]
6	2000	71	Canada	*Acremonium* spp.	[6]
5	2002	40	Venezuela	*Fusarium* spp.	[67]
4	2011	114	Argentina	*Fusarium* spp.	[25]
2	2012	59	Korea	*S. brevicaulis*	[40]
1.5	2007	78	Mexico	*S. brevicaulis*	[31]

different from other series' findings where the main causal agents were *Fusarium* spp. and *Aspergillus* spp.[21,23,29,33,34] In this study, the most frequent mould was *N. dimidiatum*, present in 93 women (52%) and 85 men (47%), which has a similar distribution between genders,[35–37] but sometimes favouring men.[21,38]

Non-dermatophyte moulds are more common in adults between the ages of 30 and 50 years,[6,9,29,31,39,40] similar to the average age of patients in this study (43 years). However, 2.8% (9/317) of these patients' ages were between 9 and 17 years, the age range in which non-dermatophyte moulds onychomycosis are very rare, with only a few cases reported in the literature[31,41,42] mainly related to infection by different *Fusarium* species.[33] This finding and the rapid progression of onychomycosis, starting 1 or 2 months before the date of diagnosis in six patients infected with *N. dimidiatum* and in two patients infected with *Fusarium* spp., is in concordance with the rapid progression of onychomycosis caused by non-dermatophyte moulds reported in another study from Colombia where 46% of the patients noted nail changes between 1 month and 2 years before diagnosis was made,[43] and contradict the opinion of other authors who consider that non-dermatophyte mould onychomycosis are chronic infections beginning several years before diagnosis.[9,18,39]

Occupational exposure to environmental risk factors such as hand or foot occlusion, repetitive trauma, food handling, wearing rubber boots, closed and sports shoes,[3,14] agricultural and farm labour[44] have also been related to non-dermatophyte onychomycosis. However, there were no relationships between occupations and onychomycosis in this study, as have been described in other urban populations.[39]

Regarding lifestyle habits related to onychomycosis, direct contact with soil by walking or showering barefoot was the most important finding in this study, followed by clinical signs of tinea pedis during physical examination. Contact with soil, regardless of the occupation and place of origin of individuals studied, can be related to the finding of *N. dimidiatum* in samples of indoor wet areas and shower floors[45] with a probable anthropophilic transmission.[46] We found in this study an important number of patients with clinical signs of tinea pedis and onychomycosis by *N. dimidiatum*, but relevance of non-dermatophyte moulds as causative agents of skin lesions could be underestimated since *N. dimidiatum* and *Fusarium* spp. cause involvement of the soles and interdigital spaces that could not be distinguished from those of dermatophytes origin.[46,47] Therefore, the clinical diagnosis of tinea pedis without aetiological confirmation could lead to a misdiagnosis of *N. dimidiatum* or *Fusarium* spp. infection with risk of treatment failure and nail invasion.

Wearing occlusive footwear is common in the population where the study took place (Bogota, Colombia). In these circumstances the possibility of direct contact with soil is reduced, but at the same time the risk of onychomycosis increases, especially when wearing boots and sports footwear made with rubber and synthetic materials because these types of materials can increase foot temperature and induce hyperhydrosis, a well-known tinea pedis risk factor.[21,48] As the majority of the 317 patients were housewives and urban residents working at desk jobs, the contact with home floors may also play an important role in the spread of the disease, comparable to outdoor soil contact (geophilic transmission).[49]

Other antecedents and lifestyle habits related to non-dermatophyte moulds onychomycosis like trauma,[27,40,43,50] manicures and pedicures[43] and sports practice[21,27,43] did not have any relevance in this population since they were identified only in 11.6% of patients.

The probable relationship between non-dermatophyte moulds onychomycosis and systemic diseases causing immune suppression has its origins in the concept of moulds as secondary pathogens.[2,3,24] This relationship has been discussed and accepted by experts in different publications[51] and case reports of onychomycosis by different species of *Fusarium* in neutropenic and HIV-infected patients,[3,52] haematological neoplasms[53] and transplant patients[31,54] that warned about the risk of potentially lethal blood spread from skin and nail lesions.[23,55,56] We found 12 immunosuppressed individuals (3.7%) in this group of patients: eight have diabetes mellitus, two were taking immune-suppressive drugs (prednisolone, methotrexate and chloroquine) for systemic erythematous lupus and rheumatoid arthritis, one had major depression and another had been diagnosed with HIV infection. In the last four cases *N. dimidiatum* was isolated as a causal agent.

Although onychomycosis have been considered more frequent in HIV-infected patients,[57] the predominant pathogens are dermatophytes and yeast,[58] with a few cases of onychomycosis by *N. dimidiatum* reported in the literature.[3,52,58] We do not know what happened with the only HIV-infected patient of the group because he never returned after the aetiology agent (*N. dimidiatum*) was confirmed. However, onychomycosis by *N. dimidiatum* in HIV-infected patients should alert the dermatologist to the risk of disseminated infection (sinusitis, mycetoma, lymphangitis, osteomyelitis and lymphadenitis).[52,59]

The most prevalent systemic disease in this group of patients was chronic lower extremity venous disease (40/317), this finding could be related to the association between non-dermatophyte mould onychomycosis and hypothermia secondary to peripheral vascular disease in some cases of onychomycosis by *Scopulariopsis brevicaulis*.[60] The probable relationship between lower limb venous insufficiency and onychomycosis by *N. dimidiatum*,[49] dermatophytes, yeast[48] and other moulds[31] have also been reported in Brazil (56%), Colombia (38%) and Mexico (15%) respectively. Other systemic disorders such as hypothyroidism,[61] diabetes[3,5,9,31,43,61] and psoriasis[3,5,34] are thought to be associated with non-dermatophyte mould onychomycosis.

Non-dermatophyte onychomycosis[34,40,43,60] and dermatophyte onychomycosis[6,48] may affect one or more toenails and fingernails, and most often involve the great toenail as identified in our population. Bilateral great toenail was the most frequent place, probably because of the slow growth of toenails, being the most exposed to and in contact with the soil, the accumulation of debris in the nail folds and the increased risk of trauma, dystrophy and infection by dermatophytes,[16] characteristics that can be exploited by the moulds to invade the nail.[5,49,60] *Aspergillus* spp.,[52] *Alternaria* spp.,[9] *Fusarium* spp.,[9,62] *S. brevicaulis*[63] and *N. dimidiatum*[38] are species that have been associated with lesions in the fingernails in the international literature, mainly in thumb nails. In this study population, there were only three cases of onychomycosis in this location, with different fingernail involvement.

The most frequent nail changes caused by non-dermatophyte moulds are chromonychia, pachyonychia and onycholysis,[18,34,43] findings that were identified in most of these patients. The predominance of leuconychia and yellowish discoloration (58.7%) agrees with some reports on onychomycosis by *S. brevicaulis*, *Fusarium* spp. and *Aspergillus* spp. where the main clinical finding has been PSO associated with nail fold inflammation, leuconychia and yellow discoloration of the nail.[16,23] However, only two patients with lesions of the fingernails presented erythema and swelling of the periungual nail fold, and isolated moulds were *N. dimidiatum* and *Penicillium* spp. Melanonychia associated with infection by *N. dimidiatum*[31,38,59,64] were observed in only seven patients.

In this population, the most frequent clinical presentation was DLSO (75.7%), followed at a considerable distance by SWO (1.2%) and PSO (0.9%), such as described in similar studies.[31,34,40] Any of these clinical forms can progress to TDO,[9] as occurred in 5% of patients.

Distal and lateral subungual onychomycosis has classically been associated with infection by *Aspergillus*

Table 6 Comparison of clinical features of non-dermatophyte onychomycosis in Bogota-Colombia, with international studies.

	Causative agents of onychomycosis					
References	*Neoscytalidium dimidiatum*	*Fusarium*	*Aspergillus*	*Acremonium*	*Penicillium*	*Scopulariopsis*
Morales *et al.* [33]	DLSO, SWO, PSO, TDO Paronychia (fingernails) Yellow, white, brown and green discoloration Melanonychia	DLSO, TDO Yellow, white and brown discoloration	DLSO Yellow and white discoloration	DLSO, SWO, PSO, TDO Yellow, white and brown discoloration Melanonychia	DLSO Paronychia (fingernails)	TDO Onycholysis Yellow discoloration
Ranawaka *et al.* [44]	None	DLSO, TDO Paronychia	Melanonychia Paronychia	None	Melanonychia Paronychia	None
Hwang *et al.* [40]	None	DLSO	DLSO, SWO, PSO, TDO	DLSO	None	DLSO, PSO
Cursi *et al.* [49]	Onycholysis Pachyonychia Melanonychia Yellow and white discoloration	None	None	None	None	None
de Magalhaes *et al.* [52]	DLSO Yellow discoloration	DLSO Onycholysis Leuconychia	Yellow and black discoloration (fingernails)	None	None	None
Ungpakorn *et al.* [70]	DLSO	DLSO	None	None	None	None
Gianni *et al.* [2]	None	WSO Paronychia	DLSO	DLSO, SWO	None	DLSO
Tosti *et al.* [23]	None	PSO, DLSO Paronychia Proximal leuconychia Yellow and white discoloration	DLSO, PSO Paronychia Proximal leuconychia	DLSO White streaks extending from the distal margin to the proximal nail plate	None	DLSO, PSO Paronychia Yellow, white and orange discoloration

DLSO, distal and lateral subungual onychomycosis; SWO, superficial white onychomycosis; PSO, proximal subungual onychomycosis; TDO, total dystrophic onychomycosis.

spp.,[2,8,16] *Acremonium* spp.,[2,16,23,26,40] *Fusarium* spp.[23,26,40] and *S. brevicaulis*,[2,16,26,27,40,65,66] and more recently with *N. dimidiatum* infection,[3,16,35,49,59] the most common pathogen found in this population. WSO is associated with *Acremonium* spp.,[2,3,26] *Fusarium* spp.[3,5,9,26] and *Aspergillus* spp. infection,[9,26] and can easily be confused with PSO when accompanied by leuconychia.[17] Other authors have considered proximal leuconychia, especially when it is adjacent to the proximal nail fold, as a typical characteristic of *Fusarium* onychomycosis[5,23,67–69] mainly by *Fusarium oxysporum.*[2,16,17] The four cases of SWO that we describe here were caused by *N. dimidiatum* ($n = 3$), and *Acremonium* spp. ($n = 1$), the same aetiological agents identified in the three cases of PSO (Table 2).

On the basis of the above data, we are able to say that non-dermatophyte mould onychomycosis have different clinical manifestations,[18] which are indistinguishable from those caused by dermatophytes.[31] Therefore, the complete mycological study performed by qualified professionals in preparing and processing samples is essential for the proper recovery and identification of the aetiological agent in any patient with clinical suspicion of onychomycosis.

The prevalence of *N. dimidiatum* infection found in this population is in contrast to previous reports indicating that *Fusarium* spp. is the most frequently isolated aetiological agent of non-dermatophyte moulds onychomycosis in Colombia,[18,21,29,43] representing up to 50% of isolates of moulds in a range of 310 cases[21] and 13.8% of all onychomycosis.[29] However, *N. dimidiatum* had already been reported as the main aetiological agent of superficial mycoses by non-dermatophyte moulds, including onychomycosis, in France[38] and

Thailand,[70] and it is considered endemic in some areas of Southeast Asia, South America, the Caribbean, India and Central Africa.[59] In Europe, most of those affected by *N. dimidiatum* onychomycosis have a history of living or visiting an endemic area, mainly in France, the United Kingdom and Spain, countries with a growing number of immigrants from less developed countries.[38] However, it is likely that the true significance of *N. dimidiatum* as a human pathogen has been underestimated in South America, where there is a wide variety of fruit trees and plants that can serve as a reservoir for the mould,[38] and where average temperature ranges are an ideal condition for growth and spread of fungus, since in many cases the infection is subclinical and because of the sample collection, processing and interpretation difficulties in laboratories with little experience.

The frequency of non-dermatophyte mould onychomycosis reported here (29 cases per year) suggests that moulds are not common pathogens in the population consulting the health care institution where the study was conducted: patients from urban areas of Bogota. Each year, an average of 750 nail samples are received for mycological study in this centre, but only 3.8% of them are confirmed with a non-dermatophyte moulds infection. Although the prevalence was lower than reported in most previous studies conducted in Colombia,[21,29,30] Asia and Africa,[1,50,70,71] it is similar to the prevalence reported by other Latin American studies conducted in Mexico,[31] Argentina,[25] Brazil[72] and Venezuela.[67]

In mixed infection, defined as the isolation of any combination of dermatophytes, yeast and non-dermatophyte moulds in the same sample, the primary pathogenic agent is usually a dermatophyte or yeast, whereas non-dermatophyte moulds are regarded as contaminants and secondary invaders.[4,24,73] Although rare, mixed infections have been described in the literature[16,34,43,64,74] and their existence has been acknowledged by several authors.[3,29,75] Nevertheless, there is still great uncertainty and controversy regarding the criteria to either confirm or exclude a diagnosis of mixed infection.[15,17,29,76] This study excluded patients with a first positive culture for any non-dermatophyte mould in whose second sample a dermatophyte or a yeast was also isolated. Even so, we were able to confirm five patients co-infected with two different non-dermatophyte moulds. This finding had previously been reported by Escobar and Carmona [77] who described the simultaneous isolation of *N. dimidiatum* and *Fusarium* spp. in one patient, and *Aspergillus versicolor* and *S. brevicaulis* in another.[21]

Although the association between the clinical presentation of onychomycosis and some species of moulds has been well described in the literature (Table 6), our results support the view that dermatophyte onychomycosis are clinically indistinguishable from those caused by moulds, as considered by some authors.[17,21,31,64,76,78] Therefore, complete mycological study (KOH and culture) should always be performed to identify the causative agent before starting any treatment.

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