

Non-dermatophyte mycelial fungi in onychodystrophies. Experience of a private medical center in the City of Buenos Aires

Ricardo Negroni, Alicia Arechavala, Pablo Bonvehí¹

Abstract

Background. In a former study, we observed a significant number of onychodystrophies caused by non-dermatophyte mycelial fungi.

Objectives. The purpose of the present study is to prove actual existence of onychomycosis by non-dermatophyte mycelial fungi, in performing at least two mycological examinations per patient.

Methods. A total of 317 nail scrapings were examined; direct microscopic examination with 40% KOH showed septated hyaline hyphae, and non-dermatophyte mycelial fungi were isolated from cultures. These patients were called for a second mycological examination.

Results. Only in 116 cases (36.6%), was a second sample possible. 60 were women, and 56 men, all of them adults between 25 and 81 years of age (mean 49.5 ys.); 13 appeared on fingernails, and 103, on toenails. In 75 cases (64.7%), the second sample confirmed the result of the first, with the following genus and species: *Fusarium* spp. 44; *Acremonium* spp 22; *Scopulariopsis brevicaulis* 5; *Paecilomyces lilacinus* 2; *Geotrichum candidum* 1, and *Aspergillus ochraceus* 1. Three of these cases may be considered mixed infections by *Trichophyton rubrum* associated with *Acremonium* spp. in two cases, and with *Scopulariopsis brevicaulis* in one. In 41 cases (35.3%), isolations of both clinical samples did not match; 23 second-sample cultures were negative, 10 grew a dermatophyte, and in 8 cases another fungus was isolated.

Conclusions. It was possible to fulfill minimum diagnosis criteria for non-dermatophyte mycelial fungi onychomycosis in 75 patients. No gender prevalence was observed. All patients were adults, most frequently affected on toenails, and especially on hallux. No specific clinical feature was detected and a clear dominance of *Fusarium* spp. (58.6 %) was found (Dermatol Argent;14(2):118-123).

Key words: onychomycoses, non-dermatophyte mycelial fungi, superficial mycoses.

Introduction

Onychomycoses are defined as nail plate fungal infections. They comprise between 30 and 60 percent of nail pathologies, with an incidence of about 1.7 and 8.4 percent of individuals. These figures exceed 20 percent after 60 years of age.¹⁻⁶

Three types of microorganisms produce fungal nail infections: dermatophytes, of uncontested pathogenic role; yeasts; and non-dermatophyte mycelial fungi (NDMF). The latter comprise a heterogeneous group of micomycetes of controversial pathogenic role, since they may behave as contaminants, colonizing, or pathogens.⁷⁻¹⁴

Various authors proposed indispensable minimum requirements to consider an onychomycosis as produced by a NDMF: 1) hyphae and/or spores in direct microscopic examination of the clinical sample, and 2) isolation of the same fungal species in a second nail sample, obtained some days later, and after extreme hygiene measures.^{7,9,15-18}

Other suggested relevant criteria are the evidence of fungal invasion of nail plate by demonstration of PAS-positive hyphae in histopathological tests of distal nail clippings, and the absence

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1. Centro de Estudios Micológicos. Autonomous City of Buenos Aires, Argentine Republic

Correspondence

Ricardo Negroni: José E. Uriburu 1252. 1º piso "C" (1114) Autonomous City of Buenos Aires – Argentine Republic.

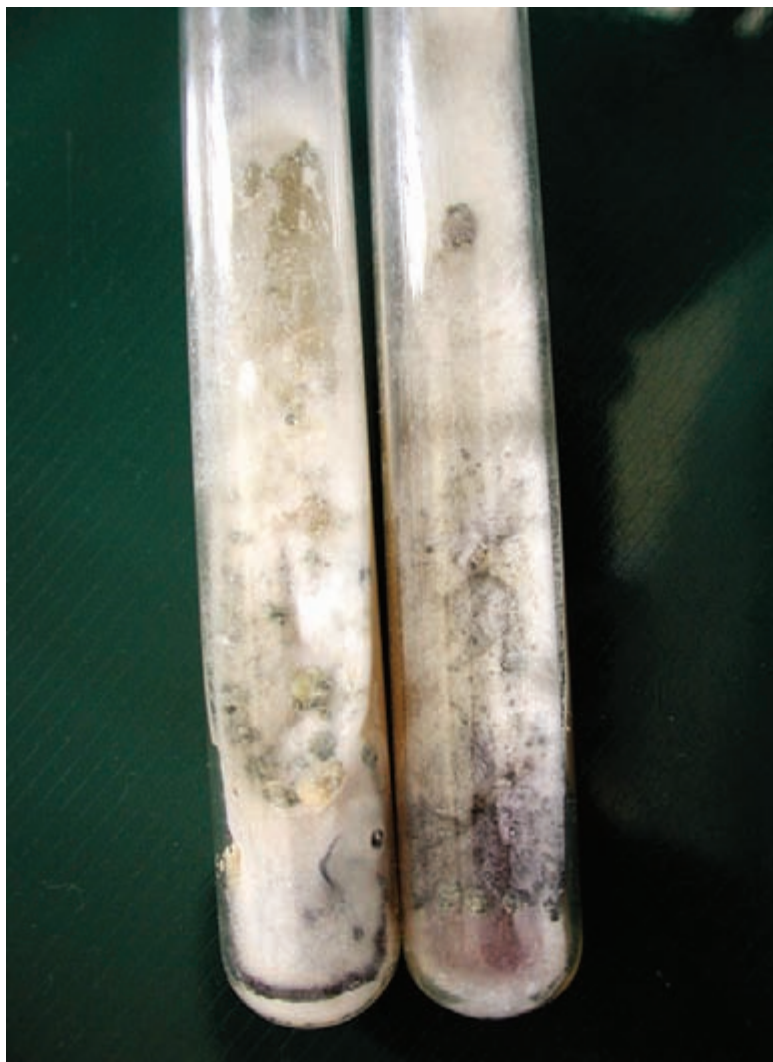


Figure 1. Gross examination of *Fusarium* spp. colonies in Sabouraud and lactrimel media cultured with toenail samples.

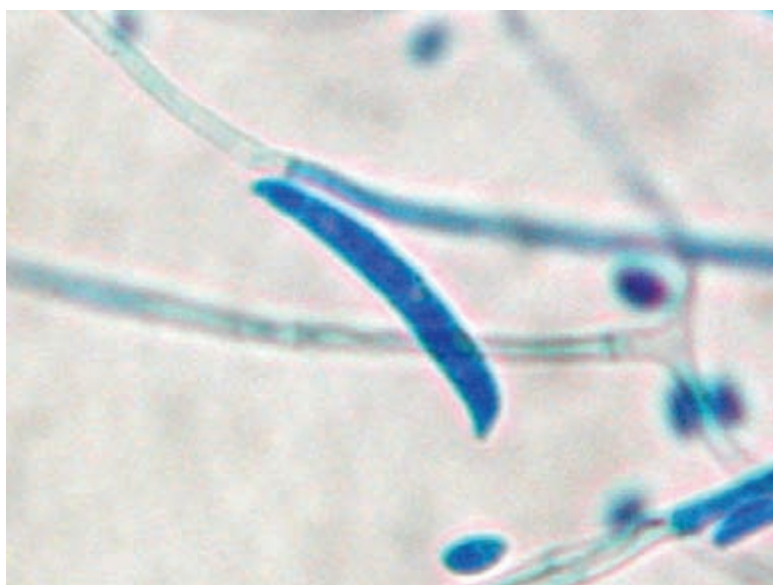


Figure 2. *Fusarium* spp. macroconidia in lactofenol preparation '400.

of dermatophyte recovery in cycloheximide media cultures.^{7,9,12} This last condition is not required by all experts, and some accept 10 to 15 percent of mixed infections, with an increasing trend.¹⁵⁻¹⁷ In these cases, it is relevant that NDMF grows in over 50 percent of the inoculation points.¹⁷

In a study conducted in the Centro de Estudios Micológicos on onychomycosis, 4.2 percent of the clinical nail samples showed significant growth of NDMF and the presence of hyaline and septated hyphae in direct microscopy examination.¹⁹ This is rather a high incidence for a non endemic area for the *Scytalidium dimidiatum* fungus, an agent causing onychomycosis in tropical areas.^{10,20-22}

The purpose of this investigation was to intend to fulfill the minimal requirements to reach diagnosis of onychomycosis by NDMF in all cases with positive direct microscopy examination for mycelial fungi and growth in cultures of one of these fungus genera.

Materials and methods

The hundred seventeen patients with positive direct microscopic examination for hyaline hyphae and NDMF growth in cultures in the absence of dermatophyte growth, were called for taking a second clinical sample.

A second sample was obtained in only 116 cases (36.6%), 13 from fingernails and 103 from toenails; in 47 of the latter patients only hallux nails were affected. All were adults, with ages between 25 and 81 (mean age: 49.5 years); 60 were women.

The clinical presentations observed were onycholysis, subungual distal and lateral invasion, superficial white, deep proximal lesions, alone or associated with inflammation of the proximal nail fold without purulent secretion.

Patients received instructions to suppress every antifungal systemic or topical treatment 15 days before the clinical sample was taken; not using powders, creams, or nail polish; to brush nails with soap and water at least twice a day on the three days before the sample was taken; and come to the Center with closed shoes and stockings, in order to prevent environmental contamination.

Specimens of nail scrapings were subjected to direct microscopy examination with 40 percent potassium hydroxide mixed 50:50 with permanent blue-black Parker® ink. The clinical sample and the solution were placed between a slide and a coverslip, and examined with light microscope at '200 and '400 for 10 minutes.¹⁹

The sample was cultured with Nikron wire hook in culture media in test tubes, marking culture points on the surface every 5 mm. Each sample was cultured in three culture media: Sabouraud's agar-honey (honey 40 g, peptone 10 g, yeast extract 5 g, agar 20 g, water qs. 1000 ml) with cloramphenicol-colistin; Borelli's lactrimel (honey 10 g, wheat flour 10 g, milk 200 ml, agar 18 g, water qs. 1000 ml) with the same antibiotic mixture, and Mycosel® (BBL, Becton-Dickinson, USA.). Cultures were incubated at 28°C for 3 weeks. Identification of genera and species was carried out by macro- and micro-morphological features of the colonies, according to Medical Mycology manuals and atlas.^{23,24}

Patients included in this study were members of high fee prepaid medical systems, and from middle or high social class, residing in the City of Buenos Aires or neighbor cities.

Results

Fungi isolated in the first nail samples from the 317 patients are listed in **Table 1**.

Of the 116 patients appearing for the second clinical nail sample, confirmation of the first specimen resulted in 75, thus fulfilling the minimum diagnosis requirement. Isolated fungal genera and species are shown in **Table 2**. Three cases were considered mixed infections since the second clinical sample showed dermatophyte growth associated with the NDMF growth in more than 50 percent of the cultured points.

The result of the second sample did not match the first one in 41 cases; cultures were negative in 23 cases, showed development of *Trichophyton rubrum* in 9, of *Trichophyton tonsurans* in 1, and of other non-dermatophyte fungi, different from the ones identified in the first sample, in the remaining 8.

As regards clinical features of onychomycosis, 51 cases were rated as onycholysis, 30 as subungual distal-lateral-type lesions, 16 as superficial white, 15 as proximal subungual, and in the 4 remaining this lesion was associated with paronychia without purulent secretion. In this last type of lesion, the isolated fungus was always *Fusarium* spp. (Figures 1 and 2). No relation was found, except in the stated case, between clinical alterations and a specific fungal genus or species.

Discussion

In the last decades, there has been an increase of NDMF onychomycosis incidence and, at the same time, unequivocal evidence of existence has been obtained.^{1,2,6,9,10,14,16,17,22} Culture isolation of one of these microorganisms from clinical nail samples may have different meanings: sometimes it is simply an environmental contamination, in others it appears as a transient or permanent colonizer of an ungual damage of another origin; and, finally, it may cause onychomycosis or complicate a nail dermatophytosis, thus causing a mixed infection.^{1,2,9,16,17} In order to clarify the role played by

TABLE 1. GENUS IDENTIFICATION OF THE 317 FUNGI ISOLATED IN THE FIRST CLINICAL NAIL SAMPLE.

Isolated fungi	Number of cases
<i>Fusarium</i> spp.	148
<i>Acremonium</i> spp.	94
<i>Aspergillus</i> spp.	25
<i>Scopulariopsis</i> spp.	17
<i>Paecilomyces</i> spp.	5
<i>Curvularia</i> spp.	3
<i>Alternaria</i> spp.	3
<i>Scedosporium</i> spp.	2
<i>Bipolaris</i>	2
<i>Penicillium</i> spp.	2
<i>Geotrichum</i> spp.	2
<i>Epicoccum</i> spp.	1
<i>Verticillium</i> spp.	1
<i>Trichoderma</i> spp.	1
<i>Scytalidium</i> spp.	1
<i>Chrysosporium</i> spp.	1

TABLE 2. IDENTIFICATION OF FUNGI ISOLATED FROM THE TWO CLINICAL NAIL SAMPLES. A TOTAL OF 75 CASES.

Fungus	Number of cases
<i>Fusarium</i> spp.	44
<i>Acremonium</i> spp.	22*
<i>Scopulariopsis brevicaulis</i>	5*
<i>Paecilomyces lilacinus</i>	2
<i>Geotrichum candidum</i>	1**
<i>Aspergillus ochraceus</i>	1

References: *2 cases with growth of *Acremonium* spp. and 1 with *Scopulariopsis brevicaulis* also showed development of *Trichophyton rubrum*. **This case showed onycholysis of fingernail.

these fungi in nail lesions, diverse requirements have been suggested to consider them as the cause of onychomycosis. These requirements were stated in the introduction, and are not easy to carry out in daily practice; thus, the first two are deemed indispensable. In this study, we intended to fulfill this minimal condition, but we were only successful in that 36.6 percent of patients called for a second sample. The obtained results enabled us to verify confirmation of the first clinical sample result in 64.6 percent of cases; thus, these fungi may be deemed causing agents of pure or mixed infections, or permanent colonizers. In 23 of the 41 cases (56 percent), where the second sample result did not match, cultures were negative, and 8 showed growth of another microorganism; this leads to the conclusion that in 26.7 percent of the cases there was a transient colonization or environmental contamination. Finally, in 10 patients (8.2 percent) only dermatophytes were recovered in the second sample and, therefore, there was an error in the first test. Studies from various places worldwide show that NDMF account for between 1.47 percent and 22 percent of the nail sample isolations.^{2,6,8,10,11,20,21,25-27} Most frequently recovered genera in Europe, North America and Mexico are *Scopulariopsis* spp., *Aspergillus* spp., and *Fusarium* spp.^{8,10,11,13,14,26-28}

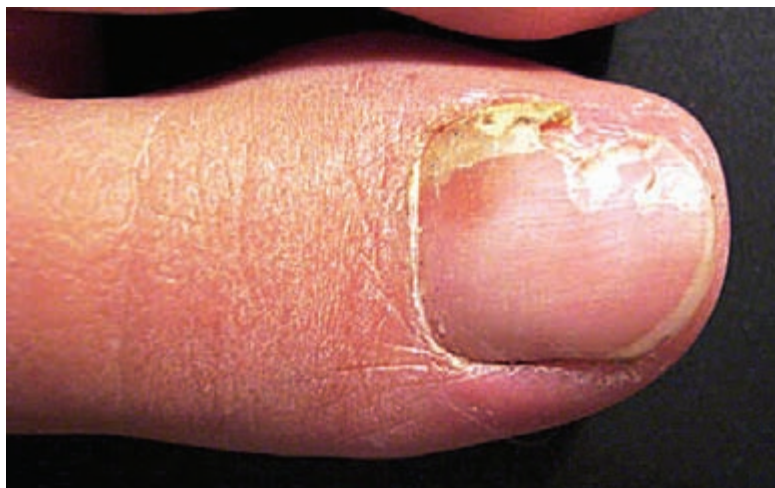


Figure 3. Subungual lateral onychomycosis caused by *Fusarium* spp. in a young 25-year-old woman without predisposing factors.

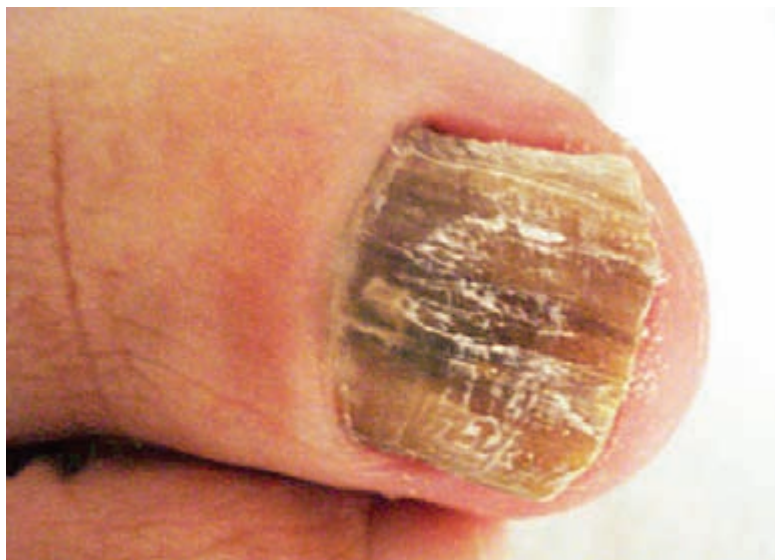


Figure 4. Deep onycholysis of hallux nail with hematoma in a 63-year-old woman with orthopedic trauma, where *Aspergillus flavus* was isolated twice.

In contrast, in this country and in Pakistan, Brazil and Colombia, dominance of *Fusarium* spp. and *Acremonium* spp was found.^{20,21,25,29,30}

Some of the NDMF causing nail lesions seemingly have a greater prevalence in certain geographical areas, such as *Scytalidium dimidiatum* in tropical areas, *Onychocola canadensis* in Canada, and *Aspergillus versicolor* in Spain.^{10,28,31} *Aspergillus terreus* has been indicated as an emerging species causing onychomycosis in elderly individuals with peripheral circulatory disorders.^{2,6,9}

In this study, *Fusarium* and *Acremonium* genera accounted for 88 percent of confirmed isolations, relegating *Scopulariopsis brevicaulis* to a third place, with only 5.3 percent of recovered cultures (Figure 3). These differences may be related to a greater or lesser presence of these microorganisms in the environment, as well as the population habits (type of shoe, sports practice, etc.).^{1,6}

Most studies indicate that HMHD onychomycosis are more frequent on toenails, particularly in hallux, and that they affect adults of both sexes and are very rare in children.^{6,7,9,11-13,20,21,26,27,32} In our study, all patients were adults, with a mean age of 49.5, with no significant differ-

ences related to gender; 88.7 percent of the cases had lesions in toenails; and of these, 45.6 percent involved only nails of the big toes. This may imply that the trauma factor plays an important role on the infection onset.

Clinical pattern of NDMF onychomycosis is variable; indicated are subungual distal and lateral type lesions, superficial white, and deep proximal lesions, occasionally associated with non-suppurative paronychia.^{7,8,10,11,14} All these lesions plus onycholysis were found in this study, thus evidencing the difficulty in clinically suspecting this fungal infection. The only exception was the 4 patients with deep proximal invasion and paronychia, where *Fusarium* spp. was isolated. However, other authors found the same pattern in nail infections caused by *Aspergillus* spp. (Figure 4).^{12,28,33}

The only case of onychomycosis with repeated isolation of *Geotrichum candidum* deserves special consideration, with fingernail onycholysis. This fungus is not the usual causing agent of nail infections; it is frequently found as dairy contaminant, and as colonizer of the digestive tract, commonly in contact with the fingernails.

Our purpose is to go deeper into the study of *Fusarium* spp. onychomycosis through histopathological studies of distal nail clippings and identification of the most frequently involved species.

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References

1. Baran R, Hay R, Haneke E, Tosti A. Onychomycosis. The current approach to Diagnosis and Therapy. 2nd. Edition. Boca Raton, FL, USA: Taylor & Francis Group; 2006.
2. Ghannoum M, Hajjeh R, Scher R, et al. A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution and antifungal susceptibility pattern. *J Amer Acad Dermatol* 2000;43:641-648.
3. Midgley G, Moore MK, Cook JC, Phan QG. Mycology of nail disorders. *J Amer Acad Dermatol* 1994; 31:568-574.
4. Roberts DT. Prevalence of dermatophyte onychomycosis in the United Kingdom. Results of an omnibus survey. *Brit J Dermatol* 1992; 126(Suppl. 39):23-27.
5. Sais G, Juclá A, Peyrí J. Prevalence of dermatophyte onychomycosis in Spain: a cross-sectional study. *Brit J Dermatol* 1995; 132:758-761.
6. Torres-Rodríguez JM, López-Jodrá O. Epidemiology of nail infections due to keratinophilic fungi. In: Kushwaha RKS, Guarro J. *Biology of Dermatophytes and other keratinophilic fungi*. Rev Iberoamer Micol 2000. pp. 122-135.

7. André J, Achten G. Onychomycoses. *Intern J Dermatol* 1987; 26:481-490.
8. Bonifaz A, Cruz-Aguilar P, Ponce RM. Onychomycosis by molds. Report of 78 cases. *Eur J Dermatol* 2007; 17:70-72.
9. Elewski B. Onychomycosis: Pathogenesis, Diagnosis and Management. *Clin Microbiol Rev* 1998; 11:415-429.
10. Ellis DH, Watson AB, Marley JE, Williams TG. Non-dermatophytes in onychomycosis of the toenails. *Brit J Dermatol* 1997; 136:490-493.
11. García-Martos P, Domínguez I, Marín P, Linares M, Mirá J, Calap J. Onicomycosis causada por hongos filamentosos no-dermatofitos. *Enferm Infecc Microbiol Clin* 2000; 18:319-324.
12. Gianni C, Romano C. Clinical and histological aspects of toenail onychomycosis caused by *Aspergillus* spp: 34 cases treated with weekly intermittent terbinafine. *Dermatology* 2004; 209:104-110.
13. Madrenys-Brunet N, Torres-Rodríguez JM, Urrea-Arbeláez A. Estudio epidemiológico de las micosis ungueales en Barcelona. *Rev Iberoamer Micol* 1996; 13:14-17.
14. Tosti A, Piraccini BM, Lorenzi S. Onychomycosis caused by nondermatophytic molds: clinical features and response to treatment of 59 cases. *Amer Acad Dermatol* 2000; 42:217-224.
15. English MP. Comment. Nails and fungi. *Brit J Dermatol* 1987; 94:481-490.
16. Summerbell RC, Kane J, Krajden S. Onychomycosis, tinea pedis and tinea marmorata caused by non-dermatophytic filamentous fungi. *Mycoses* 1989; 32:609-619.
17. Summerbell RC, Cooper E, Bunn U, Jamieson F, Grupta AK. Onychomycosis: a critical study of techniques and criteria for confirming the etiologic significance of non-dermatophytes. *Medical Mycology* 2005; 43:39-59.
18. Vander Straten MR, Hossain MA, Ghannoum MA. Cutaneous infections dermatophytosis, onychomycosis and tinea versicolor. *Infect. Dis Clin N Amer* 2003; 17:87-112.
19. Arechavala A, Bonvehí P, Negroni R. Perfil de las onicomycosis basado en 2106 exámenes micológicos. *Dermatol Argent* 2006; 12: 205-212.
20. Alvarez MI, González LA, Castro LA. Onychomycosis in Cali, Colombia. *Mycopathologia* 2004; 158:181-186.
21. Bokhari MA, Houssain I, Jahangir M, Haroon TS, Aman S, Khurshid K. Onychomycosis in Lahore, Pakistan. *Intern J Dermatology* 1999; 38:591-595.
22. Richardson M, Warnock DW. Chapter 7. Mould infections of nails. In: *Fungal Infections. Diagnosis and Management*. 2nd. Edition. Blackwell Science. Oxford, London, Edinburgh. 1993. pp. 104-106.
23. Lacaz C. da S, Porto E, Heins-Vaccari E, Takahashi de Melo N. Guia para identificação de Fungos, Actinomicetos, Algas de interesse médico. São Paulo, Brasil: Sarvier Editora de Livros Médicos Ltda; 1998.
24. Larone DH. *Medically Important Fungi. A guide to identification*. 4th. Edition. Washington DC, USA: ASM Press; 2002.
25. Garg A, Venkatesh V, Singh M, Pathak KP, Kaurhal GP, Agrawal SK. Onychomycosis in central India: clinicoetiologic correlation. *Intern. J Dermatol* 2004; 43:498-502.
26. Mercantini R, Marsalla R, Moretto D. Onychomycosis in Rome. Italy. *Mycopathologia* 1996; 136:25-32.
27. Romano C, Gianni C, Difonzo EM. Retrospective study of onychomycosis in Italy: 1985-2000. *Mycoses* 2005; 48:42-44.
28. Torres-Rodríguez JM, Madrenys-Brunet N, Sidat M, López-Jordá O, Jiménez T. *Aspergillus versicolor* as cause of onychomycosis. Report of 12 cases and susceptibility testing to antifungal drugs. *J Eur Acad Dermatol Venereol* 1998; 11:25-31.
29. Luque A, Biasoli M, Alvarez D. Aumento de la incidencia de micosis superficiales producidas por hongos del género *Fusarium*. *Rev Iberoamer Micol* 1995; 12:65-67.
30. Pontes ZB, Lima Ede O, Oliveira NM, Dos Santos JP, Ramos AL, Carvalho MF. Onychomycosis in Joao Pessoa City, Brazil. *Rev Argent Microbiol* 2002; 34:95-99.
31. Gupta AK, Hogan-Bell CB, Summerbell RC. Onychomycosis associated with *Onychocola canadensis*: ten cases report and review of the literature. *J Amer Acad Dermatol* 1998; 39:410-417.
32. Romano C, Pappini M, Ghilardi A, Gianni C. Onychomycosis in children: a survey of 46 cases. *Mycoses* 2005; 48:430-437.
33. Mahmoudabadi AZ, Zarrin M. Onychomycosis with *Aspergillus flavus*, a case report from Iram. Pakistan, *J Med Sciences* 2005; 21:497-498.