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**NON-DERMATOPHYTE MOULDS AS SKIN AND NAIL FOOT
MYCOSIS AGENTS: *PHOMA HERBARUM*, *CHAETOMIUM
GLOBOSUM* AND *MICROASCUS CINEREUS*.**

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Short summary

In this report three environmental filamentous fungi, *Phoma herbarum*, *Chaetomium globosum*, and *Microascus cinereus* were isolated and identified from immunocompetent subjects skin and nail samples.

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Summary

The increased prevalence of dermatomycoses along with the wide range of organisms now recognized as potential pathogens needs accurate laboratory isolation and identification of the aetiological agents. In this report three cases of foot dermatomycoses due to filamentous fungi commonly present in the environment with ubiquitous distribution are described in immunocompetent subjects. Skin and nail samples were collected, suspended in 20% KOH solution, examined under a light microscope and cultured in Mycobiotic agar and Sabouraud dextrose agar containing chloramphenicol to detect fungal growth. Three non-dermatophyte moulds, *Phoma herbarum*, *Chaetomium globosum*, and *Microascus cinereus* were isolated and identified.

Introduction

Cutaneous mycosis is differentiated into dermatophytosis and dermatomycoses. Dermatophytosis is a type of dermatomycoses caused by dermatophytes (Abdelrahman *et al.* 2006; Aghamirian & Ghiasian 2000). Dermatomycoses is caused by a wide variety of yeasts and non-dermatophytes fungi, with tinea like infections of the skin, hair and nails (Bonifaz *et al.* 2007; Hay 2005; Veer *et al.* 2007). Traditionally non-dermatophyte filamentous fungi have been considered as contaminant or secondary pathogens of the skin and nails; however, some of them may behave as primary pathogens (Bonifaz *et al.* 2007; Iorizzo *et al.* 2007). The prevalence of foot mycosis and onychomycosis by non-dermatophytes moulds among the population is still underestimated even if, in relation to the recent advances in diagnosis and risk factor identification, reports by the mycological medical community have increased (Iorizzo *et al.* 2007; Shemer *et al.* 2008).

In this report three cases of foot dermatomycoses due to environmental filamentous fungi in immunocompetent subjects are reported.

Methods of laboratory investigation

Skin and nail samples were subjected to direct microscopy and examined directly under a light microscope to detect fungal elements. The samples were inoculated into Mycobiotic agar (MYC; Merck, Germany) and Sabouraud dextrose agar containing chloramphenicol (SAB+C; Sigma, St. Louis, Mo) to detect dermatophytes and other types of fungi, respectively. The plates were incubated at 25°C for at least 15 days, to give any dermatophyte, that might be present, the opportunity to grow, and checked twice a week for any evidence of growth. After 2 weeks of incubation cultures were all negative for dermatophytes and yeasts and positive for non-dermatophyte moulds. Fungal cultures on SAB+C were initially sent for identification to

the Bacteriology and Mycology Laboratory, Department of Public Health and Microbiology, University of Turin, Turin, Italy. Subcultures were made on SAB agar and Oatmeal agar (Sigma) and incubated for at least 2 weeks at 25°C. Identification of moulds was based on macroscopic and microscopic characters of the colonies (performed with a 400X optical microscope). The pathogenic significance of fungal agents was confirmed by repeating cultures to exclude cases of contamination: a total of three consecutive cultures of the specimens was taken three times from the same patients with one-week intervals (De Hoog & Guarro 2000; Shemer *et al.* 2008). Final identifications were made on the basis of ITS sequences and morphology by the Centraal Bureau voor Schimmelcultures (CBS) Utrecht, Netherlands. All isolated moulds (*Phoma herbarum*, *Chaetomium globosum* and *Microascus cinereus*) are maintained in the Fungal Collection of the Bacteriology and Mycology Laboratory, Department of Public Health and Microbiology, University of Turin, Turin, Italy.

Report of cases

Patient 1

P.P., a 36-year-old healthy white female was admitted to the Medico-Surgical Discipline Department, Dermatological Clinic II, San Lazzaro Dermatologic Hospital, University of Turin. Clinical diagnosis of distal lateral onychomycosis was made. The onychomycosis involved the big toe of the left foot; the nail was hyperkeratotic, thickened, crumbly and uniformly white in the superficial face, without brownish *striae* of discoloration. No other skin diseases were present. Microscopic examination of the nail in 20% KOH solution was positive for branching septate, hyaline hyphae and did not show any dermatophyte features. Macroscopic examination of the subcultures on SAB and Oatmeal agar after a 15-d incubation at 25°C showed a fluffy colony with a white-grey obverse, with black granulation tissue, arranged in concentric rings and a brown reverse (**Fig. 1A**).

Microscope examination of the colony showed the presence of hyaline, septate hyphae; pyriform and brown pycnidia (asexual fruiting bodies 118-265 μm in length by 105-250 μm in width; **Fig. 1B**); unicellular, hyaline and oval-shaped conidia (4-8 μm by 2.5-4 μm ; **Fig. 1C**); macroscopic and microscopic examinations allowed the identification of *Phoma spp.* (*Coelomyces*). Microscopic examination and the three consecutive cultures of specimens were all positive. The resolution of the nail disease occurred after a six-month topic therapy performed with allylamine and sertaconazole. After treatment KOH preparations and cultures were negative.

The isolated mould morphologically identified as *Phoma spp.* was sent to the Centraal Bureau voor Schimmelcultures (CBS), Utrecht, Netherlands for final identification. The isolate was identified as *Phoma herbarum* Westend by the ITS sequence 100% identical to *P. herbarum* CBS 502.91 and morphology.

Patient 2

F.P., a 46-year-old healthy white male was admitted to the Alba-Bra Hospital, ASL CN2, Alba (Cuneo, Italy). He presented interdigital erythematous-desquamative itchy lesions and dry skin on the right foot. No other skin diseases were present. Microscopic examination of the skin samples in 20% KOH solution was positive for branching light brown, septate hyphae. Macroscopic examination of the subcultures on SAB and Oatmeal agar after 15-d incubation showed some pale brown colonies with brown granulation tissue and a light brown reverse (**Fig. 2A**). Microscope examination of the colonies showed globose to subglobose dark-brown perithecia (250-260 μm by 180-190 μm) with numerous septate, flexuous ascomatal hairs 100-105 μm by 2.6-4 μm (**Fig. 2B**). Ascospores were olive-brown lemon-shaped (5.6 μm by 9 μm), and contained a sub-apical single germ pore (**Fig. 2C**).

Macroscopic and microscopic examinations allowed the identification of *Chaetomium* spp. (Ascomycota). Microscopic examination and the three consecutive cultures of specimens were all positive. In view of the laboratory results, a therapy with oral terbinafine (Lamisil®) and topical tioconazole (Trosyd®) was initiated. After 15 days, skin lesions healed. On this occasion KOH preparations and cultures were negative.

The isolated mould morphologically identified as *Chaetomium* spp. was sent to the CBS for final identification. The isolate was identified as *Chaetomium globosum* Kunze ex Fr. *sensu lato* by the ITS sequence 100% identical to *C. globosum* ATCC 6205 (GenBank - EF524036.1).

Patient 3

C.M., a 57-year-old healthy white female was admitted to the San Lazzaro Dermatologic Hospital. Clinical diagnosis was distal subungual onychomycosis, involving two nails of the left foot. Nail plates were yellow with brownish discoloration and thickened. Nail beds were thickened, because of subungual hyperkeratosis. No other skin diseases were present.

Microscopic examination of the nails in 20% KOH solution was positive for branching dematiaceous, septate hyphae. No spores were seen in the specimens. Macroscopic examination of the subcultures on SAB and Oatmeal agar after 21-d incubation showed an irregular, warty, velvety, dark grey colony obverse, with a brown reverse (**Fig. 3A**). Microscopic examination of the colony showed the presence of hyaline, septate hyphae; dark globose perithecia, singly and in clusters (100-180 µm by 131-160 µm) with short cylindrical necks and dark ascomatal hairs (**Fig. 3B**). Ascospores showing convex shape were 3-5 µm by 2.6 µm. Rare anelloconidia, occurring singly or in chains, arising from penicillate conidiophores and measuring 4-5 µm by 3-5 µm were also observed. Macroscopic and

microscopic examinations allowed the identification of *Microascus cinereus* (Ascomycota), an anamorph of *Scopulariopsis cinerea*. Microscopic examination and the three consecutive cultures of specimens were all positive. No therapy was performed because the patient refused any treatment.

All isolated moulds (*P. herbarum*, *C. globosum* and *M. cinereus*) are maintained in the Fungal Collection of the Bacteriology and Mycology Laboratory, Department of Public Health and Microbiology, University of Turin, Turin, Italy.

Discussion

The prevalence of non-dermatophytic fungi implicated in foot mycoses varies in the literature (De Hoog & Guarro 2000). The most prevalent non-dermatophytic fungi associated with foot dermatomycoses and onychomycosis are *Aspergillus spp.*, *Acremonium spp.*, *Scopulariopsis spp.* and *Fusarium spp.*. More rarely *Curvularia spp.*, *Scytalidium spp.* and *Penicillium marneffeii* are involved (Bonifaz *et al.* 2007; Gupta *et al.* 2007; Jesudanam *et al.* 2002). In this report we describe other filamentous fungi.

Phoma spp., *Chaetomium spp.* and *Microascus spp.* are ubiquitous environmental phytopathogens present in the soil, air and on plants.

Phoma belongs to the class *Coelomycetes* and is characterized by colonies with dark spherical pycnidia with single, or occasionally multiple ostioles. Conidia are hyaline to pale coloured, small, mostly unicellular and oval-spherical in shape (De Hoog & Guarro 2000).

The genus *Chaetomium* belongs to the class *Euascomycetes* and is characterized by black perithecia. The ascospores are aseptate, smooth-walled, pigmented, with one or two germ pores (De Hoog & Guarro 2000).

Microascus also belongs to the *Euascomycetes* and is characterized by perithecia which are usually black, spherical to pyriform and may have very short to noticeably long

cylindrical necks. Ascospores are aseptate, smooth-walled and variable in shape depending on the species (De Hoog & Guarro 2000).

Cases of human infections ascribed to *Phoma spp.*, *Chaetomium spp.* and *Microascus spp.* have been rarely reported by the medical mycology community. *C. globosum* and *M. cinereus* have both been reported as the etiologic agents of human nail infections (Agarwal & Singh 1980; Aspiroz *et al.* 2007; Hattori *et al.* 2000). Moreover, *M. cinereus* has emerged as a significant invasive pathogen in immunocompromised patients (Baddley *et al.* 2000); it is better known and frequently recovered as a *Scopulariopsis* anamorph in more superficial settings and an etiological agent (Bonifaz *et al.* 2007; Iorizzo *et al.* 2007). *Phoma spp.* are frequent agents of lesions in different plants; but animals and insects may also be parasitized. Human lesions caused by species of the genus *Phoma* may be superficial or deep, attacking the skin, cornea, subcutaneous cell tissue and lungs. *Phoma spp.* has been isolated from cutaneous and subcutaneous lesions of the feet but there is no prior evidence of involvement in onychomycosis (Zaitz *et al.* 1997).

Laboratory examination is very important to confirm the diagnosis of dermatomycoses and onychomycosis because the clinical appearance caused by one species of fungus is often indistinguishable from that caused by other fungi (Shemer *et al.* 2008). In addition, the existence of environmental filamentous fungi that cause infections underlines the need to not disregard them as contaminants but to study these moulds as potential pathogens (Tullio *et al.* 2008).

The set criteria for the diagnosis of mycosis due to non-dermatophytic moulds are: 1) observation of fungal elements in 20% KOH- preparations made from nail and skin scraping; 2) growth of the same mould in all three consecutive cultures of specimens taken three times from the same patient with one-week intervals; 3) no growth of a dermatophyte or yeast in three consecutive cultures (English 1976; McGinnis 1980).

Moulds isolated from the three subjects we have described are considered pathogenic either because the strains grew pure in cultures and from all specimens or because the criteria, suggested by McGinnis (1980) and English (1976) to establish the aetiology of fungal infection, are met in these three case reports. Hence, we consider the filamentous fungi *P. herbarum*, *M. cinereus* and *C. globosum* as causative agents of foot infections in immunocompetent individuals. To the best of our knowledge, this appears to be the first documented report of human onychomycosis caused by *P. herbarum*.

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References

- Abdelrahman T, Letscher Bru V, Waller J, Noacco G, Candolfi E, 2006. Dermatomycosis: comparison of the performance of calcofluor and potassium hydroxide 30% for the direct examination of skin scrapings and nails. *Journal of Mycological Medicine* **16**: 87-91.
- Agarwal GP, Singh M, 1980. *Microascus cinereus* infection of human nail. *Indian Journal of Medical Science* **34**: 263-265.
- Aghamirian MR, Ghiasian SA, 2008. Dermatophytoses in outpatients attending the Dermatology Center of Avicenna Hospital in Qazvin, Iran. *Mycoses* **51**: 155-160.
- Aspiroz C, Gene J, Rezusta A, Charlez L, Summerbell R, 2007. First Spanish case of onychomycosis caused by *Chaetomium globosum*. *Medical Mycology* **45**: 279-282.

- Baddley JW, Moser SA, Sutton DA, Pappas PG, 2000. *Microascus cinereus* (Anamorph *Scopulariopsis*) brain abscess in a bone marrow transplant recipient. *Journal of Clinical Microbiology* **38**: 395-397.
- Bonifaz A, Cruz-Aguilar P, Ponce RM, 2007. Onychomycosis by molds. Report of 78 cases. *European Journal of Dermatology* **17**: 70-72.
- de Hoog GS, Guarro J, Gene J, Figueras MJ and Guarro GJ, 2000. Atlas of Clinical Fungi, 2nd edn. Utrecht, the Netherlands: Centraalbureau voor Schimmelcultures.
- English MP, 1976. Nails and fungi. *British Journal of Dermatology* **94**: 697-701.
- Gupta M, Sharma NL, Kanga AK, Mahajan VK, Tegta GR, 2007. Onychomycosis: Clinico-mycologic study of 130 patients from Himachal Pradesh, India. *Indian Journal of Dermatology, Venereology and Leprology* **73**: 389-392.
- Hay R, 2005. Literature review. Onychomycosis. *Journal of the European Academy of Dermatology and Venereology* **19**: 1-7.
- Hattori N, Adachi M, Kaneko T, Shimozuma M, Ichinohe M, Iozumi K, 2000. Case report. Onychomycosis due to *Chaetomium globosum* successfully treated with itraconazole. *Mycoses* **43**: 89-92.
- Iorizzo M, Piraccini BM, Tosti A, 2007. New fungal nail infections. *Current Opinion in Infectious Disease* **20**: 142-145.
- Jesudanam TM, Rao GR, Lakshmi DJ, Kumari DL, 2002. Onychomycosis: a significant medical problem. *Indian Journal of Dermatology, Venereology and Leprology* **68**: 326-329.
- McGinnis MR, 1980. Reported fungal pathogens of man. Appendix C. In: *Laboratory handbook of medical mycology*. Academic Press, New York, pp. 619-643.

- Shemer A, Davidovici B, Grunwald MH, Trau H, Amichai B, 2009. New criteria for the laboratory diagnosis of non-dermatophyte moulds in onychomycosis. *British Journal of Dermatology* **160**: 37-39.
- Tullio V, Mandras N, Banche G, Allizond V, Gaido E, Roana J, Cuffini AM, Carlone NA, 2008. *Schizophyllum commune*: an unusual agent of bronchopneumonia in an immunocompromised patient. *Medical Mycology* **46**: 735-738.
- Veer P, Patwardhan NS, Damle AS, 2007. Study of onychomycosis: prevailing fungi and pattern of infection. *Indian Journal of Medical Microbiology* **25**: 53-56.
- Zaitz C, Heins-Vaccari EM, de Freitas RS, Arriagada GL, Ruiz L, Totoli SA, Marques AC, Rezze GG, Müller H, Valente NS, Lacaz Cda S, 1997. Subcutaneous phaeohyphomycosis caused by *Phoma cava*. Report of a case and review of the literature. *Revista do Instituto de Medicina Tropical de São Paulo* **39**: 43-48.

Figure legends

Figure 1. Macroscopic and microscopic morphology of *Phoma herbarum* after 15-d incubation on SAB: granulation tissue of the colony (**A**); pyriform brown pycnidium (**B**; 400X) and oval shaped conidia (**C**; 400X).

Figure 2. Macroscopic and microscopic aspect of *Chaetomium globosum* after 15-d incubation on SAB: pale brown colonies (**A**); dark brown globose perithecium with dark flexuous hairs (**B**; 400X); lemon-shaped ascospores (**C**; 630X).

Figure 3. Macroscopic and microscopic features of *Microascus cinereus* after 21-d incubation on SAB: dark grey, warty and irregular colony (**A**); dark globose perithecium with a short cylindrical neck (**B**; 400X).

Fig. 1

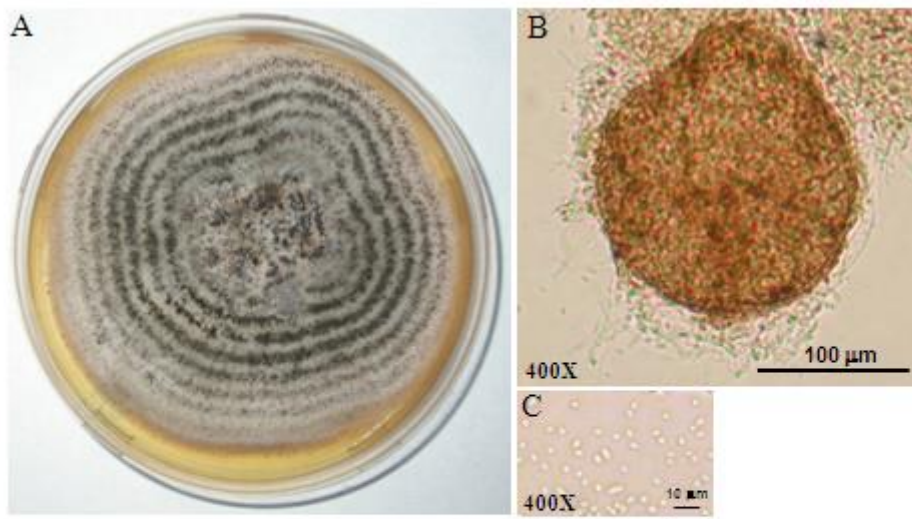


Fig. 2

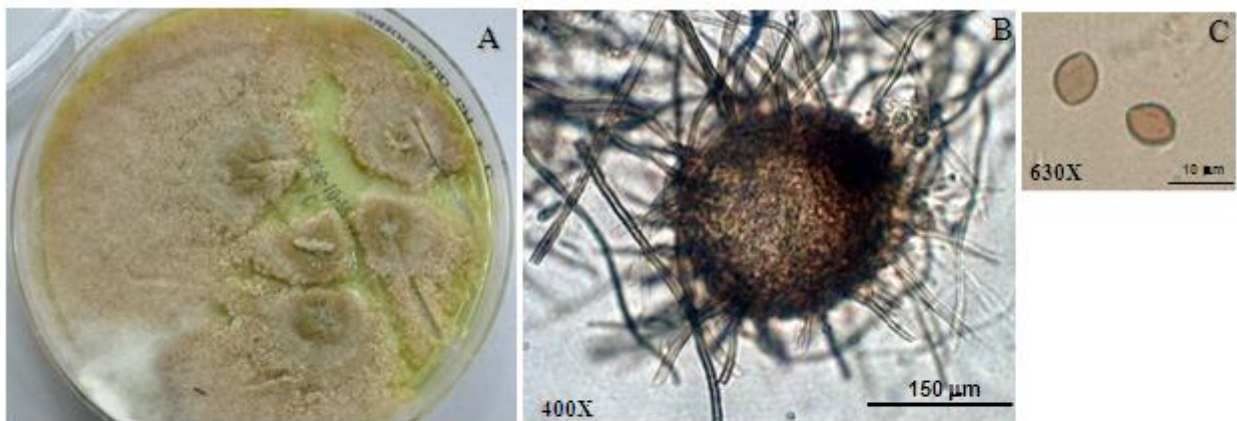


Fig. 3

