

Diagnosis of *Tinea pedis* and onychomycosis in patients from Portuguese National Institute of Health: a four-year study

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ABSTRACT: *Tinea pedis* and onychomycosis are two rather diverse clinical manifestations of superficial fungal infections, and their etiologic agents may be dermatophytes, non-dermatophyte moulds or yeasts. This study was designed to statistically describe the data obtained as results of analysis conducted during a four year period on the frequency of *Tinea pedis* and onychomycosis and their etiologic agents. A questionnaire was distributed from 2006 to 2010 and answered by 186 patients, who were subjected to skin and/or nail sampling. Frequencies of the isolated fungal species were cross-linked with the data obtained with the questionnaire, seeking associations and predisposing factors. One hundred and sixty three fungal isolates were obtained, 24.2% of which composed by more than one fungal species. Most studies report the two pathologies as caused primarily by dermatophytes, followed by yeasts and lastly by non-dermatophytic moulds. Our study does not challenge this trend. We found a frequency of 15.6% of infections caused by dermatophytes (with a total of 42 isolates) of which *T. rubrum* was the most frequent species (41.4%). There was no significant association ($p > 0.05$) among visible injury and the independent variables tested, namely age, gender, owning pet, education, swimming pools attendance, sports activity and clinical information. Unlike other studies, the variables considered did not show the expected influence on dermatomycosis of the lower limbs. It is hence necessary to conduct further studies to specifically identify which variables do in fact influence such infections.

Keywords: *Tinea pedis*, onychomycosis, frequency, etiologic agents, variables.

Diagnóstico da *Tinea pedis* e onicomicose em pacientes do Instituto Nacional de Saúde em Portugal: estudo de 4 anos

RESUMO: *Tinea pedis* e onicomicose possuem uma grande diversidade nas suas formas clínicas. Os seus agentes etiológicos podem ser fungos dermatófitos, fungos filamentosos não dermatófitos ou leveduras. Este estudo foi desenvolvido com o intuito de caracterizar estatisticamente os resultados provenientes de um estudo desenvolvido durante quatro anos sobre a frequência de *Tinea pedis* e de onicomicose e dos respetivos agentes etiológicos. Foi distribuído um questionário de 2006 a 2010, tendo o mesmo sido respondido por 186 pacientes, que foram submetidos a colheita de pele e/ou unhas. A frequência dos isolados das espécies fúngicas foi relacionada com os dados provenientes do questionário, procurando associações com fatores que se conhecem como favorecedores das infeções. Foram obtidos 163 isolados de espécies fúngicas, em que 24,2% apresentavam mais do que uma espécie. A maior parte dos estudos, que incidem nesta temática, referem os fungos dermatófitos como os agentes etiológicos mais comuns, seguidos pelas leveduras e pelos fungos filamentosos não dermatófitos. O nosso estudo corroborou os mesmos resultados. Os fungos dermatófitos apresentaram frequência de 15,6% (com um total de 42 isolados), em que o *T. rubrum* foi a espécie mais frequente (41,4%). Não se verificou associação significativa ($p > 0,05$) entre lesão visível e as variáveis independentes testadas, designadamente: idade, sexo, animal de estimação, educação, frequência de piscinas, atividade física e informação clínica. Ao contrário de outros estudos, as variáveis testadas não apresentaram a influência esperada nas dermatomicoses dos membros inferiores, sendo por isso necessário realizar mais estudos para identificar as variáveis que influenciam ambas as infeções.

Palavras-chave: *Tinea pedis*, onicomicose, frequência, agentes etiológicos, variáveis.

Introduction

According to the *Achilles Project*, developed in sixteen European countries in 2003, 34.9% of 70497 subjects had fungal infections in their feet, being both *Tinea pedis* and onychomycosis the most common affections. *Tinea pedis*, commonly known as "athlete foot", is an inflammatory condition and the most common form of dermatomycosis. It can be transmitted either by direct contact between two individuals or, indirectly, through contaminated objects or surfaces¹. Onychomycosis is usually associated with *Tinea pedis* and may occur due to trauma in the nail during physical activity². Both diseases can occur due to exposure to contaminated surfaces with bare feet².

There is a wide diversity of clinical forms from *Tinea pedis* and onychomycosis. Their etiologic agents may be dermatophytes, non-dermatophyte moulds (NDM) and yeasts. Most of the authors diagnose dermatophytes as the most frequent etiological agents (80 to 90%), followed by yeasts (5 to 17%) and finally by NDM (2 to 12%)^{1,3}.

In Portugal, according to the Mycology Laboratory of the National Institute of Health (2007), the number of analysis to fungal infections in keratinized tissues is increasing. In fact, during 2002, 250 mycological exams to keratinized products whereas in 2006 more than the double of exams (near 600) were performed. From the ones performed during 2006, *Tinea pedis* and onychomycosis cases (48.0%) were caused by dermatophytes and the remaining 52.0%, by yeasts and NDM. It was also found that, in the same year, the two most frequently isolated dermatophyte species were *Trichophyton rubrum* and *T. mentagrophytes*.

Given the growing relevance of the topic, this investigation was designed to:

- statistically describe data obtained from 2006 to 2010 in what concerns to *Tinea pedis* and onychomycosis and their etiologic agents, and also;
- to explore possible associations with available variables commonly referred to as risk factors for these two diseases.

Materials and Methods

A study was conducted in National Institute of Health during the period 2006 to 2010. Patients selected for this study were the ones sent from dermatology consultation with suspected fungal infection in skin or nails. A questionnaire was distributed and answered by 186 patients who were subjected to feet skin and/or nails sampling by medical doctor's request. This questionnaire was composed of four distinct sections, namely: the identification of the individuals and their characteristics, including age, gender, scholar qualifications and ownership of pets as the first section. The second focus on leisure activities, including swimming and sporting that could contribute to an increased fungal exposure. Third section sought to obtain patients' clinical information that could predispose a fungal infection, such as diabetes, psoriasis or cancer, and also other information able to influence laboratory's diagnosis,

as previous treatment with antifungal drugs. The fourth section concerned information about the lesion (visible or not) and its location within the body. This section was filled out by the technicians who collected the samples. The questionnaire also presented an informed consent from the patients to participate in this study.

The sampling was performed in one hundred eighty six patients with suspected lesion, using a sterile scalpel to collect skin or nail scrappings to a sterile Petri dish. Subsequently, residual scales were collected with a sterile swab pre-moistened with saline solution. Direct microscopic examination of the samples was performed following treatment with potassium hydroxide (KOH, 30%), during no less than 20 minutes. Samples were inoculated onto Sabouraud dextrose medium supplemented with chloramphenicol (agar and broth) and mycobiotic agar, supplemented with chloramphenicol and cyclohexamide. The inoculated media were incubated at 25° C for a period of 15 to 20 days^{4,5}.

Identification of filamentous fungi was carried out using microscopic preparations with lactophenol blue staining and achieved through the observation of morphological characteristics listed in illustrated literature⁶. Yeasts were identified through biochemical test from bioMérieux⁷. Whenever possible, filamentous fungi were identified to the species level, since adverse health effects vary according to fungal species⁸.

Direct observation of fungal elements and abundant colony-formation of single species were considered factors for validation of the etiological agent.

Tables with distribution frequencies of isolated fungal species were constructed with the data obtained and possible associations of fungal infection with studied variables asked in the questionnaire were also analyzed. To compare quantitative variables between two independent groups, we used the t test, to assess the association between qualitative variables we used the frequency analysis and Chi-square test.

Confidentiality of the obtained results was assured in order to ensure the protection of the provided information.

Results

Patient's characteristics

One hundred eighty six patients were enrolled in this study (patients presenting lesions suspected to be caused by fungal infection). From those, sixty one (32.8%) were male and 125 (67.2%) were female. The mean age of the patients was 45 years. About 48% of the patients had higher education. 44.6% of the patients were swimming pools users and 59.9% sported regularly, other than swimming. The pet animals most frequently owned were dogs (31.0%) followed cats (16.3%). One hundred and forty seven patients (79.0%) had visible lesions on their feet and the most frequently affected area was the toenail (83.0%). During the biological sampling collection, 42

patients (22.6%) were carried out an antifungal treatment (cf. Table 1).

Table 1: Patient's characteristics

Patient's characteristics	Total
Male	61
Female	125
Higher education	89
Swimming pools usage	83
Sported regularly	111
Dog as a pet	58
Visible lesions	147
Toenail affected	154
Antifungal treatment	42

Laboratorial diagnosis

From the samples analyzed, 163 fungal isolates were obtained and in 24.2% of the cases more than one fungal species were isolated ("mixed" infections). Dermatophytes were isolated 15.6% (with a total of 42 isolates) being *T. rubrum* the most frequently isolated species (41.4%, cf. Table 2). In addition to *T. rubrum*, *T. mentagrophytes*, *T. tonsurans* and *Epidermophyton sp.*, other species were also identified, namely: *M. audouinii*, *M. canis*, *M. gypseum*, *T. violaceum*, *T. verrucosum*, *T. erinacei* and *T. sholeinii*.

Table 2: Dermatophytes, NDM and Yeasts isolation frequency

	No. of positive samples	(%)
Dermatophytes	42	15.6
<i>T. rubrum</i>	17	41.4
<i>T. mentagrophytes</i>	5	17.2
<i>T. tonsurans</i>	3	13.8
<i>Epidermophyton sp.</i>	2	6.9
Others	15	20.7
NDM	121	44.6
<i>Aspergillus sp.</i>	23	14.4
<i>Fusarium sp.</i>	10	10.0
<i>Scytalidum sp.</i>	8	7.8
Others	80	67.8
Yeasts	108	39.8
<i>Candida sp.</i>	71	71.2
<i>Rhodotorula sp.</i>	30	16.9
<i>Cryptococcus sp.</i>	4	6.8
Others	3	5.1
Total	271	100

As NDM, the genus *Aspergillus* was the most frequently isolated genus, with a total of 23 (14.4%) isolates (cf. Table 1). Besides *Aspergillus*, *Fusarium* and *Scytalidum* genera, others were also isolated with some expression: *Penicillium*

sp., *Cladosporium sp.*, *Arthrinium sp.*, *Alternaria sp.* and *Paecilomyces sp.*

Candida was the most frequently isolated yeast, with a total of 71 (71.2%) isolates (cf. Table 1). *Candida parapsilosis* was the most prevalent species represented by 40.5% of the total of *Candida* isolates.

Several patients' variables were analyzed in order to explore possible associations and determine possible risk factors (cf. Table 3). No significant association ($p > 0.05$) was found between visible injury and the independent variables tested, namely with age, gender, pet owning, education, swimming pools usage, sporting and other clinical data (cf. Table 3).

Table 3: Results of the chi-square test to evaluate the association between visible injury and independent variables tested and t test to compare age between visible injury

	Independent variables	χ^2 or t	df	p value
Visible injury	Age	1.026*	249	0.306
	Gender	2.542**	1	0.11
	Owning pets	0.010**	1	0.92
	Level of education	2.432**	5	0.787
	Swimming pools usage	0.005**	1	0.94
	Sporting	1.023**	1	0.31
	Clinical information	0.563**	1	0.45

*Statistics of t test; **Statistics of Qui-square test

Discussion

Since 1980 there has been a dramatic increase in fungal infections occurrence, mainly due to the increase of population at risk⁷. Dermatophytosis affects a large segment of the world population and had enormous influence on health until the mid-twentieth century. These infections may have not been extensively studied since they were considered, incorrectly, more as an esthetic problem than a health problem⁹. Most of these infections may appear as epidemics in populations, especially in children and teenager populations.

Onychomycosis affects approximately 2.8%¹⁰ to 5% of world population¹¹. It occurs more often in toenails than in fingers, which can be due to several aspects, including: the slower nail growth, facilitating the pathogenic fungal activity of the species involved; less effective circulation in the lower limbs; and also the high trauma incidence in toenails due to footwear and physical activity¹².

In 1997, Gupta, Jain and Lynde¹³ in Canada, conducted a study in 2001 dermatology patients and found the prevalence of onychomycosis to be 9.1%. According to Arenas-Guzmán¹⁰, this disease constitutes about 50.0% of the studied onychopathies, with a prevalence of 26.9% in Europe. In Denmark, in a study also carried out to obtain the prevalence of onychomycosis, it was found that onychomycosis show a prevalence of 4.14%¹⁴.

In another study, this one conducted in Germany in 2000, called *Foot Check Study*, prevalence of onychomycosis was observed to be 12.4%, while 31.6% of study participants had *Tinea pedis* and/or onychomycosis¹⁵.

In the present study, onychomycosis was detected in 83.0% of the samples analyzed, which was expectedly higher than in the general population because those patients had already a suspected fungal infection.

The frequency of isolation of dermatophytes was only 17.2% in our study. However, most of the authors diagnose dermatophytes as the most frequent etiological agents (80 to 90%), followed by yeasts (5 to 17%) and finally by NDM (2 to 12%)^{1,3}. This can be explained by the high number of patients under antifungal treatment. Those patients should be subjected to other biological sample collection, without being under antifungal treatment, to confirm the negativity of the result.

Regarding dermatophytes, *T. rubrum* was the most frequently found species (41.4%), which is in concordance with other international and national studies¹⁶⁻²⁰.

Our study confirmed the results obtained by another four year survey (2002 to 2006 – unpublished), as well as, and studies from other authors²¹. *T. mentagrophytes* was the second species most frequently isolated (17.2%) According to the later authors and given that *T. rubrum* and *T. mentagrophytes* have the same ecological characteristics; the former species is the most frequently found because it has better capacity to adapt than *T. mentagrophytes*. Moreover, *T. mentagrophytes* produces inflammatory lesions that may heal spontaneously²¹.

Concerning specifically Portuguese studies in the field, *T. rubrum* is referred as the most frequent agent of dermatomycosis (around 50%)¹⁹. The most significant regional variations were found in relation to the second most frequently isolated species: *M. canis* in districts of Braga and Porto¹⁸⁻¹⁹, *Trichophyton megninii* in the district of Coimbra²¹ and *T. mentagrophytes* in Lisbon²²⁻²³. However, in a study developed by Teles and Rosado (1989), in 123 automobile industry workers from Setúbal, *T. mentagrophytes* was isolated in 31% of the workers whereas *T. rubrum* was isolated in only 15%²⁴.

In the present study NDM were the most isolated fungi, being also the most diverse group. These fungi can be disseminated in the environment, such as *Alternaria*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium* and *Phoma*²⁵, but are also considered as skin contaminants. Direct observation of fungal elements and abundant colony-formation of single species were considered factors for validation of the etiological agent. Repeat cultures were not possible in this study in order to confirm the etiological agent. Onychomycosis frequency by NDM ranges from 4.5 to 39.6% in different studies²⁵⁻²⁸. Other studies show that nails invasion by these fungi is considered unusual with prevalence's ranging from 1.5% to 17.6%²⁹. Prevalence variation may reflect geographic differences in fungal distribution, criteria differences in used for diagnosis of onychomycosis, and also use of laboratory methods unsuit-

able for fungal growth²⁸. Nevertheless, in recent years, the increased number of onychomycosis cases caused by NDM, particularly *Fusarium* sp. and *Aspergillus* sp., required the inclusion of such fungi as possible etiologic agents of these diseases²⁶. Furthermore, several *Scopulariopsis* species are also known to cause opportunistic infections, and *S. brevicaulis* is a well-known agent of onychomycosis²⁰. We have found only one case of onychomycosis caused by these species.

Among NDM, the most common species causing onychomycosis are *Fusarium solani* and *F. oxysporum*, which also produce other diseases such as dermatomycoses and systemic infections²⁶.

According to Godoy, Nunes and Silva³⁰, *F. oxysporum* is the most often associated *Fusarium* species in onychomycosis.

Aspergillus is the most common NDM genus in this study (14.4%), being *A. versicolor* the most frequently isolated species. Both *Scytilidium* and *Fusarium* genus were also found in our study with a frequency of 7.8% and 10.0% respectively. These are capable of metabolizing nails' keratin, but with less efficiency than the dermatophytes. This metabolic ability is not equal, however, for all *Scytilidium* species²⁶.

Concerning yeasts, *Candida* genus was the most frequently isolated (71.2%). Several aspects may contribute to *Candida* infections, namely the immune system mitigation due to age and the nail injury and subsequent infection caused by dermatophytes, which subsequently may also facilitate infection by yeasts²⁹. *Rhodotorula* spp and *Candida parapsilosis* were the most frequently isolated (16.9% and 40.5%, respectively). In a study performed by Meireles, Rocha and Brillhante³¹, *C. parapsilosis* was the second most isolated yeasts species being considered by other studies³²⁻³³ as the most frequent agent isolated from toenail onychomycosis.

More than one fungal species were isolated from 24.2% of the patients. Although uncommon, mixed infections may occur especially due to the presence of fungi that grow faster than dermatophytes³⁴. Previously, mixed infections were diagnosed incorrectly, because there was a widespread tendency to ignore NDM. More recently, however, several studies focused on mixed infections incidence. According to Gupta, Cooper and MacDonald³⁵ mixed infections should be considered when NDM are isolated in one or more occasions, and also the same dermatophyte and NDM. As reported by Gupta, Cooper and MacDonald³⁵, in our study, it was not also possible to perform more than one sampling at different time frames, as suggested in some studies^{29,36}.

There was no significant association ($p > 0.05$) amongst visible injury and independent variables tested, including age, gender, owning pets, level of education, swimming pools usage, sporting and collected clinical data.

Despite our study not finding positive associations between visible injury and patient's age, some studies reported an increased prevalence of fungal infections

alongside with age³⁷. Furthermore, younger patients are usually subjected to antifungal treatment at an earlier stage of disease in opposition to what is observed in patients over 55 years⁵.

In the present study no significant association ($p > 0.05$) was found between gender and visible injury. Nevertheless, several studies^{13,17} show that *Tinea pedis* and *Tinea unguium* are more frequently isolated in men than in women, possibly due to differences in geographic locations or number of patients analyzed. Similar contrast was observed for onychomycosis^{36,38-39}.

No association was found between owning pets and visible injury on patients despite references to dermatomycosis being the most common skin diseases in pets⁴⁰. This should facilitate the transfer of infection from animals to humans⁴¹.

In this study there was no positive association with education level. Nevertheless, a positive association was found in a study by Szepietowski, Reich and Garlowska³, which shows that individuals with higher scholar education had a lower prevalence of both pathologies, as low education levels tends to lead to more precarious working conditions.

Regarding swimming pool usage and sporting, no positive association was found presence of visible injury on patients, despite being widely described as risk factors for *Tinea pedis* and onychomycosis^{39,42}. The presented study aimed to analyze the casuistry of *Tinea pedis* and onychomycosis in patients from Portuguese National Institute of Health for a four year period. We cannot exclude the existence of associations between several variable and visible injury since the studied population is probably too small to reach to the "statistically significant" result. Nevertheless, the presented study is relevant to alert the clinicians and researchers to local epidemiology.

Conclusions

In the present study, the frequency of fungal isolation was 83.0% among patients sent from dermatology consultation with suspected fungal infection in skin or nails. *T. rubrum* was the most frequently found species which is in concordance with other international and national studies. However, and unlike reported in other studies, the variables analyzed in our study did not show influence on dermatomycosis of the lower limbs.

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