

A 10-YEAR SURVEY OF DERMATOMYCOSES IN UBERABA, SOUTHEAST BRAZIL

LEVANTAMENTO DE 10 ANOS DE DERMATOMICOSSES EM UBERABA, SUDESTE DO BRASIL

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ABSTRACT

Superficial fungal infections, known as dermatomycoses, are among the main dermatological pathologies and affect approximately 25% of the world's population. Knowing the epidemiology and promoting the diagnosis of these mycoses is essential for proper treatment administration. A total of 2,325 skin, nail and hair samples were collected from all patients treated at a private clinical laboratory in the city of Uberaba - Minas Gerais - with clinical suspicion of dermatomycosis between August 2012 and July 2022 and identified using classical methodology and the VITEK®2 YST ID system. Of the collected samples, 1,327 (57.10%) were positive for fungi, of which the majority were nail samples (61.64%), followed by skin samples from the feet (14.92%) and body (11.75%). Of these, the majority were from patients between the ages of 31 and 40 years (18.23%), and the majority of scalp samples were obtained from patients up to 10 years of age (91.66%). The most frequent fungi were dermatophytes (48.61%), followed by yeasts (28.79%) and non-dermatophyte filamentous fungi (NDFF) (22.61%), and the most common species were *Trichophyton rubrum* (41.22%), *Candida parapsilosis* (13.34%), *Candida albicans* (7.69%) and *Aspergillus* sp. (6.63%). The data herein corroborate the results of the majority of studies found in the literature and highlight the importance of knowing the prevalence of the etiological agents of these mycoses so that the correct treatment can be administered and, consequently, cases of empirical prescription, recurrence and antifungal resistance can be avoided.

KEYWORDS: superficial mycoses, dermatophytes, epidemiology, non-dermatophyte filamentous fungi, superficial candidiasis.

RESUMO

As infecções fúngicas superficiais, conhecidas como dermatomicoses, estão entre as principais patologias dermatológicas e afetam aproximadamente 25% da população mundial. Conhecer a epidemiologia e promover o diagnóstico dessas micoses é essencial para a administração adequada do tratamento. Foram coletadas 2.325 amostras de pele, unhas e cabelos de todos os pacientes atendidos em um laboratório clínico privado na cidade de Uberaba - Minas Gerais - com suspeita clínica de dermatomicose entre agosto de 2012 e julho de 2022 e identificadas por meio de metodologia clássica e pelo sistema VITEK®2 YST ID. Das amostras coletadas, 1.327 (57,10%) foram positivas para fungos, sendo a maioria de amostras de unhas (61,64%), seguidas por amostras de pele dos pés (14,92%) e do corpo

(11,75%). Destas, a maioria era de pacientes entre 31 e 40 anos (18,23%), e a maioria das amostras de couro cabeludo foi obtida de pacientes com até 10 anos de idade (91,66%). Os fungos mais frequentes foram dermatófitos (48,61%), seguidos por leveduras (28,79%) e fungos filamentosos não dermatófitos (FFND) (22,61%), e as espécies mais comuns foram *Trichophyton rubrum* (41,22%), *Candida parapsilosis* (13,34%), *Candida albicans* (7,69%) e *Aspergillus* sp. (6,63%). Os dados aqui encontrados corroboram os resultados da maioria dos estudos encontrados na literatura e ressaltam a importância de se conhecer a prevalência dos agentes etiológicos dessas micoses para que o tratamento correto possa ser administrado e, conseqüentemente, casos de prescrição empírica, recidiva e resistência antifúngica possam ser evitados.

PALAVRAS-CHAVE: micoses superficiais, dermatófitos, epidemiologia, fungos filamentosos não dermatófitos, candidíase superficial.

INTRODUCTION

Superficial fungal infections, known as dermatomycoses, are mycoses that affect structures derived from the epidermis, such as skin, hair and nails, and affect both immunocompetent and immunosuppressed individuals of all ages, sexes and ethnicities, being among the main dermatological pathologies¹. They are the most common mycoses, with an estimated prevalence of 20 to 25% among the world population and may reach 50% among individuals aged 70 years or older².

These fungal infections can be caused by yeasts, such as those of the genus *Candida*, by filamentous dermatophytic fungi, classically represented, despite taxonomic updates, by the genera *Trichophyton*, *Epidermophyton* and *Microsporum*, and by non-dermatophyte filamentous fungi (NDF), such as *Aspergillus*, *Scopulariopsis brevicaulis* and *Fusarium*¹. Of these, *Trichophyton rubrum* is the most prevalent³, but others have gained prominence in recent years, especially *Candida* species⁴.

Although dermatomycoses are clinically painless, they promote psychological, psychosocial and socioeconomic impacts, as they directly affect the self-esteem, quality of life and interpersonal relationships of affected individuals, who feel embarrassed because of visible lesions and often ask for work leave or do not even get positions in the labour market⁵. In addition, the treatment of these mycoses is a major challenge for the medical community, especially the treatment of elderly patients, pregnant women and children⁶.

In this context, the non-identification of etiological agents together with high rates of self-diagnosis and self-medication⁷, the irregular use of antifungal drugs and long treatment

favor therapeutic failure and, consequently, relapse and an increase in the number of infections and occurrences of antifungal resistance^{8,9}.

Given this scenario and because different species and strains of fungi have different antifungal susceptibility profiles, including resistance, knowing the epidemiology of this type of infection by identifying the fungus that causes it is essential so that medical and health authorities can provide adequate, targeted and individualized prevention and treatment¹⁰. Therefore, the objective of this study was to identify the etiological agents in skin, hair and nail samples from patients with clinical suspicion of dermatomycosis over a 10-year period treated at a private clinical laboratory in the city of Uberaba, Minas Gerais, Brazil, which also serves patients from the macro-region around this city, a tropical highland climate region.

METHODS

A total of 2,325 skin, hair and nail samples were collected from all patients treated at a private clinical laboratory in the city of Uberaba - Minas Gerais - with clinical suspicion of dermatomycosis between August 2012 and July 2022. The following exclusion criteria were applied: incorrect identification of the etiological agent, impossibility of isolating the suspected etiological agent due to contamination and/or exacerbated growth of several microorganisms, report of recent use of oral and/or topical antifungal, insufficient sample amount and samples packaged in an inappropriate container or at an inappropriate temperature. This work is based on a retroactive data analysis, in which no sensitive patient information, such as identity, was exposed. In addition, since these are samples obtained from patients treated in a private laboratory, your confidential data is automatically protected by the General Data Protection Law (Law 13.709/2018) of our country, so that no personal information can be disclosed without prior authorization.

Clinical samples were collected before initiating oral and/or topical treatment. If a patient was undergoing treatment, the patient was asked to suspend, with medical authorization, the use of antifungal drugs before collection for at least two weeks for topical antifungal agents and two months for oral antifungal agents. For the collection of nails, it was cleaned with soap and water and free of nail polish, and the patient was required to

refrain from using hand or foot creams that day, as the presence of these substances could hinder and interfere with the collection. The nails to be collected were sanitized with 70% alcohol and cut with sterile scissors; detached parts that showed no signs of infection were discarded, and the deepest, powdery areas were scraped using a sterile scalpel. For the collection of hair and skin, the region was sanitized with 70% alcohol, and scraping was performed with a sterile scalpel blade, preferably collecting skin from the edge of lesions. Tonsured hair was removed with sterile forceps. After collection, all samples were placed directly in a sterile, sealed flask at room temperature. For a fungus to be considered the actual etiological agent, when there was doubt and/or more than one suspected fungus, such as a saprophyte, for example, the collection was repeated and the same fungus that grew again was considered for identification¹¹.

Direct examination was performed after bleaching the clinical samples with potassium hydroxide (KOH). Two drops of 20% KOH were placed on a new, clean and degreased slide. Using a cold flamed or sterile disposable platinum loop, a small amount of the collected biological material was placed on the slide, mixed with KOH and covered with a coverslip, exerting light pressure on the slide to remove excess liquid with absorbent paper. After approximately 20 minutes, the slide was analyzed under a light microscope with 10x and 40x objectives¹¹.

A portion of each biological sample was collected with a cold flamed or sterile disposable platinum loop and deposited on the surface of Sabouraud chloramphenicol agar (Probac[®]) and Mycosel agar (Probac[®]), pressing it to allow good contact with the culture medium. All plates were incubated at 28 °C for up to 28 days in a microbiological oven¹¹.

When fungi presented sufficient growth to be evaluated, macroscopic characteristics were analyzed, for example colony appearance, coloration of the top and reverse side, texture and pigment production. For yeast, isolated colonies were cultivated on Candida chromogenic agar (Probac[®]) incubated in a microbiological oven at 36 ± 2 °C for 72 hours. After this period, the sample was identified using the VITEK[®]2 YST ID card (BioMerieux[®]) in a VITEK 2 COMPACT 60 system (BioMerieux[®]) following the manufacturer's recommendations.

If the fungus was filamentous, one or two drops of cotton blue (lactophenol blue) were placed on a new, clean and degreased slide, and with a properly flamed and cold

platinum loop, fragments of an isolated and homogeneous colony were deposited on the stain, after which the fragments were covered with a coverslip. After a few minutes, the slide was analyzed under a light microscope with a 40x objective¹¹.

Filamentous fungi were analyzed in a potato agar block (Probac®) incubated on a slide on a support inside a glass petri dish in a humid atmosphere created with cotton moistened with physiological solution. All materials used were sterilized by autoclaving. After the block was seeded, it was covered with a coverslip, and the plate was incubated at 28 ± 2 °C for 7 days or until there was sufficient growth. Then, the coverslip was removed from the surface of the agar, mounted on a slide with one or two drops of cotton blue (lactophenol blue) and, after a few minutes, fungal structure analysis was performed under a light microscope with a 40x objective¹¹.

RESULTS

Of the 2,325 samples, the majority were nail scrapings (1,139, 49.0%), followed by skin of the feet (442, 19.0%), skin of the trunk and limbs (body) (302, 13.0%), skin of the hands (163, 7.0%), skin from the crural region (116, 5.0%), skin from the scalp (93, 4.0%) and skin from the face (70, 3.0%). The majority of samples were from females (1,299/55.90%), and the ages of the patients ranged from 1 to 98 years, with a median of 40 years (Table 1).

Table 1. Site of origin of the samples according to the age and gender of the patients.

Age (years)	Nail		Feet		Body		Hands		Crural		Scalp		Face		Total
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
0-10	0	0	1	0	23	28	0	1	2	0	12	10	0	0	77
11-20	17	33	14	8	11	13	2	3	5	3	1	0	1	2	113
21-30	57	70	31	16	11	13	4	5	13	6	0	0	4	4	234
31-40	65	80	31	17	9	12	4	4	8	4	0	0	4	4	242
41-50	63	77	25	14	8	9	4	5	6	3	0	0	2	1	217
51-60	70	95	13	7	4	5	2	3	5	2	0	0	2	1	209
61 or more	81	110	14	7	4	6	2	3	2	1	1	0	2	2	235
Total	353	465	129	69	70	86	18	24	41	19	14	10	15	14	1327
	818		198		156		42		60		24		29		

M: male; F: female.

As seen in Table 1, of the total samples, 1,327 (57.10%) were positive for fungi, of which 818 (61.64%) were nail samples, 198 (14.92%) were samples from feet, 156 (11.75%)

were samples from the body, 42 (3.16%) were samples from the hands, 60 (4.52%) were sample from the crural region, 24 (1.80%) were samples from the scalp, and 29 (2.18%) were samples from the face. Of these positive samples, the majority were from patients between the ages of 31 and 40 years (18.23%), followed by those over 60 years of age (17.70%) and those between the ages of 21 and 30 years (17.63%). Notably, most scalp samples were obtained from patients up to 10 years of age (22/91.66%), and most nail samples were obtained from patients older than 60 years (191/23.35%).

The relationship between the site of origin of the sample and the species causing the infection is shown in Table 2.

Table 2. The relationship between the site of origin of the sample and the species causing the infection.

Fungi	Anatomical site								
	Nail	Feet	Body	Hands	Crural	Scalp	Face		
<i>T. rubrum</i>	204	179	109	16	38	0	1	547	41,22%
<i>T. mentagrophytes</i>	17	15	6	2	3	0	10	53	3,99%
<i>M. canis</i>	0	0	7	1	0	11	0	19	1,43%
<i>E. floccosum</i>	1	4	0	0	6	0	0	11	0,83%
<i>M. gypseum</i>	0	0	3	0	0	3	0	6	0,45%
<i>T. tonsurans</i>	0	0	2	0	0	3	0	5	0,38%
<i>M. audouinii</i>	0	0	2	0	0	2	0	4	0,30%
Total	222	198	129	19	47	19	11	645	48,61%
<i>C. parapsilosis</i>	147	0	0	17	5	0	8	177	13,34%
<i>C. albicans</i>	86	0	0	5	6	0	5	102	7,69%
<i>Rhodotorula</i> sp.	41	0	0	0	0	0	0	41	3,09%
<i>Malassezia</i> sp.	0	0	27	0	0	5	5	37	2,79%
<i>Candida</i> sp.	11	0	0	1	2	0	0	14	1,06%
<i>C. guilliermondii</i>	6	0	0	0	0	0	0	6	0,45%
<i>C. krusei</i>	5	0	0	0	0	0	0	5	0,38%
Total	296	0	27	23	13	5	18	382	28,79%
<i>Aspergillus</i> sp.	88	0	0	0	0	0	0	88	6,63%
<i>Penicillium</i> sp.	69	0	0	0	0	0	0	69	5,20%
<i>Scytalidium</i> sp.	28	0	0	0	0	0	0	28	2,11%
<i>Aspergillus niger</i>	25	0	0	0	0	0	0	25	1,88%
<i>Cladosporium</i> sp.	25	0	0	0	0	0	0	25	1,88%
<i>Acremonium</i> sp.	21	0	0	0	0	0	0	21	1,58%
<i>Fusarium</i> sp.	7	0	0	0	0	0	0	7	0,53%
<i>Rhizopus</i> sp.	13	0	0	0	0	0	0	13	0,98%
<i>Alternaria</i> sp.	10	0	0	0	0	0	0	10	0,75%
<i>S. brevicularis</i>	8	0	0	0	0	0	0	8	0,60%
<i>Curvularia</i> sp.	6	0	0	0	0	0	0	6	0,45%
Total	300	0	0	0	0	0	0	300	22,61%
Total	818	198	156	42	60	24	29	1327	100%

A. Dermatophytes. B. Yeasts. C. NDFF: non-dermatophyte filamentous fungi.

The most frequent fungi were dermatophytes (48.61%), followed by yeasts (28.79%) and NDFF (22.61%), and the most common species were *T. rubrum* (41.22%), *Candida parapsilosis* (13.34%), *Candida albicans* (7.69%) and *Aspergillus* sp. (6.63%). The most prevalent fungi in nails were NDFF (36.67%), with the most isolated fungus being *T. rubrum*

(24.93%); in all other cases, with the exception of samples from the hands and face, in which yeasts were more frequent (54.76% and 62.0%, respectively), dermatophytes prevailed.

DISCUSSION

Dermatomycoses have a global distribution and are related to several predisposing factors, such as climatic and socioeconomic conditions, intercontinental population migration, urbanization, sports activities such as swimming, population density, prolonged and frequent contact with water, lifestyle, increased animal domestication, immunological status, use of certain medications, advancement of diagnostic techniques, greater access to health services and patient age^{4,12}.

Tropical and subtropical regions have the most favorable climatic conditions for fungal growth, with higher temperatures and humidity¹³; therefore, Brazil is one of the countries with high rates of mycoses, especially superficial cases. Nevertheless, the geographic distribution spectrum of these infections has expanded in recent years, not only in these warmer regions⁴. As they are not notifiable diseases in Brazil, it is difficult to accurately estimate the extent of the problem, reinforcing the need for periodic surveys of their frequency and etiological agents and for the assessment of socioeconomic factors and geographic, climatic and epidemiological data in different regions. These data make it possible for epidemiological and health surveillance services to promote prevention and control actions as well as allocate more resources to diagnosis and treatment¹⁴.

Dermatomycosis caused by dermatophytes (dermatophytosis) is the most common and most prevalent fungal infection among all types of mycoses worldwide, including in developing countries. Although *T. rubrum* is still the most isolated species in dermatophytosis, others such as *T. tonsurans*, *M. canis* and *M. audouinii* have emerged as the main etiological agents in some regions. In the Americas, *Trichophyton rubrum*, *T. tonsurans* and *T. mentagrophytes* are the most common species¹⁵.

Our data corroborate the results of most reports in the literature, i.e., dermatophytes are the most frequent fungi, with *T. rubrum* being the most common species, followed by yeasts, especially *Candida* spp. A study by Silva et al.¹⁶, also conducted in Uberaba (Minas Gerais/Brazil), in which 216 samples from patients with suspected dermatomycosis treated

at private and public clinics were analyzed, showed that 116 (53.70%) samples were positive for yeasts, 70 (32.40%) were positive for dermatophytes and 30 (13.90%) were positive for NDFF. *C. parapsilosis* was the most frequent species (24.10%), followed by *T. rubrum* (17.10%), *C. guilliermondii* (11.10%) and *T. interdigitale* (11.10%), highlighting the epidemiological variability in this type of infection in the same geographic region.

In addition to knowing the most frequent etiological agents, it is very important to make a survey of the most affected sites, which are clinically divided based on the anatomical site affected into tinea unguium (nails/onychomycosis), tinea capitis (scalp), tinea pedis (feet - “athlete’s foot”), tinea cruris (crural region), tinea corporis (trunk and lower and upper limbs), tinea barbae (beard), tinea faciei (face) and tinea manuum (hands)¹⁷.

In a systematic review covering 24 studies of dermatophytosis in Brazil, between 2011 and 2019, an overall prevalence of 25% was determined, with the majority of patients being women (63.9%), and the most affected sites were the nails (tinea unguium) and feet (tinea pedis). The most isolated etiologic agents were *T. rubrum* (66%), *T. interdigitale* (22%) and *T. mentagrophytes* (04%). In the cases of tinea capitis (04%), *T. tonsurans* (54%) and *M. canis* (37%) were the most frequent fungi¹⁸. Of our samples, *T. rubrum* was not more prevalent in the mycoses of the hands and face. The intention of this survey is not to assume that our results represent the general scenario of the country, but to contribute with regional data, which, together with other data from other regions, can help to elucidate the epidemiology of these infections.

In this study, onychomycosis was predominant, followed by infections of the skin of the hands and body. In the study by Silva et al.¹⁶, onychomycosis was predominant (43.10%), followed by infections of the skin of the feet and body, accounting for 22.20% each. Notably, 72.20% of infections were associated with the nails and skin of the hands or feet, and similarly, in this study, 79.72% of infections were from these same sites, further corroborating studies that report onychomycosis as the most common form of dermatomycosis.

Mycosis that affects the feet and hands is considered a risk factor for onychomycosis and usually occurs concomitantly¹⁹. The nails of the feet are most affected, and most onychomycoses (60 to 70%) are caused by *T. rubrum* and *T. mentagrophytes*. However, in recent decades, there has been an increase in the prevalence of other causative fungi, such as

S. brevicaulis, *Acremonium* spp., *Aspergillus* spp., *Fusarium* spp. and *Candida* spp.²⁰. There is a trend of increasing incidence of onychomycosis with increasing age, being much lower among children, with the lowest prevalence²¹. In this study, no nail samples were collected from children under 10 years of age; the majority (23.35%) were collected from individuals aged 60 years or older.

Importantly, although onychomycosis is viewed as a purely cosmetic condition, it represents up to 50.0% of all onychopathies, with a prevalence of 5.50% globally, and directly affects the quality of life of patients, especially those who work with food or in customer service and beauty salons, for example²². Therefore, studies such as ours contribute to a better understanding of the epidemiology of these infections, which are common among the general population but so little explored in Brazil.

The distribution of the etiological agents of scalp mycoses (very common dermatomycosis in children) in different regions of the world is complex, with *M. canis* as the most frequently isolated agent²³. Despite its prevalence, *T. tonsurans* also is an important agent of dermatomycosis in children²⁴, with 70.0% of cases occurring between 3 and 8 years of age. Our results reinforce these data, in which 91.66% of clinical isolates from the scalp originated from children up to 10 years of age; *Microsporum canis* (45.83%) was the species most isolated from the scalp, followed by *T. tonsurans* and *M. gypseum* (12.50% each).

Although we observed hegemony of dermatophytes in dermatomycoses, as in most studies, NDFF have been widely reported, especially in regions with warmer climates²⁵. For example, 3.0 to 11.0% of onychomycosis cases may be due to a mixed infection between these fungi and dermatophytes, but their roles in actually causing the infection are still not completely understood²⁶. Historically, due to their wide distribution in the environment, they have always been treated as contaminants, which makes diagnosis difficult, but they are still found in between 2.0 and 20.0% of onychomycosis cases globally²⁷.

Among the fungal identification techniques, despite advancements in molecular methods, direct microscopy and culture are still considered the gold standard, and the vast majority of laboratory services still use this classic methodology for fungal identification²⁸. Direct microscopy with KOH is an inexpensive, quick procedure that is easy to prepare, handle and perform, in addition to not requiring state-of-the-art equipment or infrastructure. However, despite allowing the visualization of fungal structures and directing identification,

this method does not allow determining fungal viability, and its sensitivity and specificity may be low²⁹.

Culture is considered the only technique capable of determining viability and identifying fungi concurrently. It allows the visualization of macroscopic characteristics typical of different species, for example, pigmentation, texture, growth rate and shape of the colonies. It is also an inexpensive and simple technique. One negative aspect is the long incubation time for some fungi, which can generate false-negative results³⁰. In addition, increasingly specific chromogenic culture media have given an enormous boost to presumptive microbial identification³¹, as have automated cards such as VITEK[®] 2 YST³².

The ideal scenario would be to combine these techniques with more refined and up-to-date ones³³, and a recognized limitation of this study is the lack of molecular identification of clinical isolates. It is important to emphasize that, since this is a private health service, the use of this type of technique is not part of the microbiological identification protocol, especially due to the high costs of execution, equipment, reagents and the need for specialized labour³⁴.

In most cases, antifungal drugs are still empirically prescribed, as diagnostic hypotheses are almost always decided by clinical examination because fungal diagnoses are still time consuming and specific tests are often not requested, potentially leading to an increase in unnecessary medication and patient follow-up costs. An Irish survey reported that confirmatory diagnostic tests were requested in only 21.9% of the 3.9 million cases of tinea capitis between 2016 and 2020 and that 38.8% of patients received only topical therapy or no antifungal treatment³⁵. It is estimated that approximately US\$ 1.67 billion is spent annually for treatment each year and that approximately 60% of general practitioners use topical steroids in the event of suspected dermatomycosis without laboratory diagnosis³⁶. This type of medication causes changes in local cellular immunity and, if prescribed and/or used incorrectly, may result in reduced infection control and, consequently, adverse effects³⁷ and the extension and recurrence of mycosis, with subsequent use of higher and longer doses of antifungal drugs to achieve an adequate therapeutic response^{38,39}.

CONCLUSION

Given all these reports of identification of the main etiological agents of dermatomycoses, it is important to have an overview of the frequency of these fungi through diagnosis because, in addition to being able to recognize the epidemiology of these infections in different regions, there is greater cost–benefit and lower expenses for health services and patients when treatment is started only after this diagnosis, including diagnoses through classic methodologies⁴⁰. Furthermore, mycological tests are essential to avoid treatment failure, incorrect diagnoses, side effects and unnecessary drug interactions and to enable targeted therapy based on the identified strain²⁹. Therefore, our epidemiological survey, despite being regional, contributes to the understanding of the profile of the etiological agents of dermatomycoses.

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CONFLIT OF INTEREST

The authors declare no conflict of interest. The Sabin laboratory is a private commercial laboratory. G.F.O is an employee of the Sabin Laboratory.

AUTHOR CONTRIBUTIONS

G.F.O. carried out all the biological assays. D.B.C.O., G.F.O. and A.G.M.G.O. analyzed the data and conceived and supervised the project. All authors wrote and approved the final manuscript.

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