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Abstract: BACKGROUND: Superficial mycoses are fungal infections limited to the outermost layers of the skin. Dermatophytic filamentous fungi and yeasts are the major causative agents of these mycoses. Dermatophytosis is one of the clinical conditions caused by fungal infections most commonly found in dermatological practice. Thus, knowledge of the ecology of dermatophytes provides a better understanding of the natural history of dermatophytosis. OBJECTIVE: This study aimed to investigate epidemiological and mycological features of superficial mycoses diagnosed from 2005 to 2011 in the Dermatology Clinic of the Hospital do Servidor Público Municipal de São Paulo, Brazil. METHOD: This retrospective study was conducted in the Laboratory of Medical Mycology at the Dermatology Clinic of the Hospital do Servidor Público Municipal de São Paulo. Mycological examinations of 9042 patients with clinical suspicion of superficial mycoses performed between 2005 and 2011 were reviewed. RESULTS: Of 9042 direct microscopic examinations, 2626 (29%) were positive for dermatophytes, 205 (2.3%) were positive for Malassezia, 191 (2.1%) were positive for other types of yeast, 48 (0.5%) were positive for bacteria, and 5972 (66%) were negative. Mean age of patients was 48 years, 6920 (77%) patients were female and 2112 (23%) were male. CONCLUSION: The biota consisted of six dermatophyte species: T. rubrum, T. mentagrophytes, M. gypseum, T. tonsurans, E. floccosum, and M. canis. The most common site of involvement was the nail and foot in adults and scalp in children, with a female predominance. Both Candida and Malassezia were more prevalent in adult women, the former most commonly affecting the interdigital region and nails and the latter the chest and neck.

Keywords: Malassezia; Tinea; Yeasts

INTRODUCTION

Superficial mycoses are fungal infections limited to the outermost layers of the skin and its appendages.1 The chief causative agents of these mycoses are dermatophytes and yeasts.

Dermatophytes are filamentous fungi able to digest and obtain nutrients from keratin and relatively insoluble high-molecular-weight proteins composed of amino acids and peptide bonds that are present in the skin, hair and nails.2 There are 39 dermatophyte species divided into three Trichophyton, Microsporum, and Epidermophyton. These species are classified as anthropophilic, geophilic or zoophilic according to their habitat. 1,3,4 Geophilic dermatophytes are soil-dwelling organisms

that grow on human or animal keratin present in the soil or on decomposing keratin sources in the environment; zoophilic organisms are found primarily in animals, while anthropophilic species are primarily associated with humans.1,3 Lesions caused by anthropophilic dermatophytes are less inflammatory than those caused by other species.4 The distribution of dermatophyte fungi varies from region to region and over time,^{2,3} being influenced by several factors, such as climatic variations, socioeconomic factors, lifestyle, presence of pets, and age.3

The most common site of involvement of dermatophytes is the skin and its appendages, and the species that affect the hair rarely affect the fingernails

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and vice versa. Dermatophyte infections can be further divided according to the affected body site into tinea capitis (scalp), tinea pedis (foot), tinea corporis (body), tinea cruris (groin), tinea manuum (hand), and tinea unguium (fingernail). 4,5 Dermatophytosis is one of the clinical conditions caused by fungal infections most commonly found in dermatological practice.6 Thus, knowledge of the ecology of dermatophytes provides a better understanding of the natural history of dermatophytosis.3 The diagnosis of dermatophytobe made by direct mycological examination with potassium hydroxide (KOH) 10% of biological material obtained from patients with suspected mycosis, providing results more rapid than fungal cultures, which may take days or weeks. This information, together with clinical history and laboratory diagnosis, ensures that the appropriate treatment is initiated promptly.

Malassezia spp. and Candida spp. are among the yeasts that cause skin infections. Malassezia spp., an agent of superficial mycosis, is strictly lipophilic and part of the normal microbiota of the skin and scalp.3 Although without keratinolytic activity, this fungus lives on the skin around the hair and uses epithelial debris or waste products as energy sources for development. 1,6 This agent is the cause of pityriasis versicolor and has been implicated in the pathogenesis of seborrheic dermatitis.6 Candida spp., a yeast component of the normal human microbiota, is considered an opportunistic yeast that compromises, individually or together, mucous membranes, skin and nails. 1,67 This fungus has a worldwide distribution and affects people of all ages, with a preference for certain professionals, such as domestic workers, laundresses, cooks, and nurses.7 Several reasons have been suggested to explain the increased incidence of yeast infections in recent decades, including the overuse of antibiotics, immunosuppressants and cytostatic drugs as well as the increasing number of AIDS patients.^{1,8}

The recognition of both types of superficial mycoses is of paramount importance due to the great frequency with which they are diagnosed in clinical dermatology. Also, these infections are contagious and responsible for epidemics in some population groups, such as tinea pedis among athletes and tinea capitis in orphanages.¹

Because superficial mycoses are not notifiable diseases in Brazil, it is difficult to accurately estimate the extent of the problem. This fact reinforces the need for periodic surveys of the frequency of fungal infections and their etiologic agents, including socioeconomic factors and geographic, climate and epidemiological data, in order to develop appropriate prevention and control measures. Thus, it is important to know the species of dermatophytes occurring in a

given region over a period of time in order to determine the most common species.

Objective

This study aimed to investigate clinical, epidemiological and mycological features of superficial mycoses diagnosed from November 2005 to July 2011 in the Dermatology Clinic of the Hospital do Servidor Público Municipal de São Paulo, Brazil.

PATIENTS AND METHODS

This retrospective study was conducted in the Laboratory of Medical Mycology at the Dermatology Clinic of the Hospital do Servidor Público Municipal de São Paulo, Brazil. Mycological examinations of 9042 patients with clinical suspicion of superficial mycoses performed between November 2005 and July 2011 were reviewed.

Six clinical variants of dermatophytosis were considered: tinea pedis, tinea unguium, tinea corporis, tinea capitis, tinea manuum, and tinea cruris.

Samples were obtained by scraping the scales with a scalpel blade (skin and nails) or removing the hair with hair tweezers. The specimens were cleared using KOH 20% with dimethyl sulfoxide (DMSO), slide-mounted and examined under direct microscopy. Cultures were obtained after seeding on Sabouraud agar and Mycosel agar plates (dermatophytes only). The colonies were identified by visual inspection of plates (macro aspects) and microscopic examination. When necessary, microculture plates and biological tests were used.

RESULTS

Of a total of 9042 direct microscopic examinations of patients with clinical suspicion of superficial mycoses, 2626 (29%) were positive for dermatophytes, with hyaline septate hyphae and arthrospores, 205 (2 3%) were positive for Malassezia spp., 191 (2.1%) were positive for yeasts, 48 (0.5%) were positive for bacteria, and 5972 (66%) were negative. Mean age of patients was 48 years, 6920 (77%) patients were female and 2112 (23%) were male (Table 1).

The mean age of patients positive for dermatophytes (n=2626) was 49 years, 1860 (71%) were female and 766 (29%) were male. Of 2626 dermatophytes isolated, 2426 (92.4%) had positive cultures, and the results were analyzed in this study (Table 2). The dermatophyte species most commonly isolated were T. rubrum (Table 2). The frequency of dermatophytes isolated in relation to different age groups is shown in table 3. Regarding the number of cases per age group, patients aged 51-60 years accounted for most cases (n=679, 28%), followed by patients aged 41-50 years (n=605, 25%) and patients aged >60 years (n=553, 23%) (Table 4). The most

frequent subtypes were tinea unguium (n=1511, 62.26%), tinea pedis (n=617, 25.42 (Table 5).

In cases positive for Candida, the mean age of patients was 50 years, 154 (81%) patients were female and 37 (19%) were male. The interdigital region and nails accounted for 171 (90%) of cases, and other regions for 20 (10%) of cases. In cases positive for Malassezia, the mean age of patients was 37 years, 125 (61%) patients were female and 80 (39%) were male. The chest and neck accounted for 141 (69%) of cases, and other regions for 64 (31%) of cases.

TABLE 1: Results of 9042 direct microscopic examinations

Direct microscopic examination	N	0/0	95%	6CI					
Negative	5972	66.0	65.1	67.0					
Dermatophyte	2626	29.0	28.1	30.0					
Malassezia spp.	205	2.3	2.0	2.6					
Yeast	191	2.1	1.8	2.4					
Bacteria	48	0.5	0.4	0.7					
Total	9042	100							
95%CI = 95% confidence interval									

TABLE 2: Dermatophyte species isolated (positive cases) – absolute and relative numbers and confidence interval

Dermatophyte species	N	0/0	95%	ωCI					
T. rubrum	2333	96.2	96.0	96.9					
T. mentagrophytes	57	2.3	2.3	3.0					
M. gypseum	15	0.6	0.6	0.9					
T. tonsurans	8	0.3	0.3	0.6					
E. floccosum	7	0.3	0.3	0.5					
M. canis	6	0.2	0.2	0.4					
Total	2426	100							
95%CI = 95% confidence interval									

DISCUSSION

Dermatophytosis remains a common clinical condition in humans worldwide. Factors such as weather conditions, social practices, and hygiene practices certainly contribute to the epidemiological variations in dermatophytes.

When all dermatophyte species isolated from 2426 dermatophyte-positive cultures were analyzed, the predominance of T. rubrum followed the international and national trends and this agent was also the most frequent across all age groups (Table 3).²⁸⁻¹³ This fungus is considered a major etiologic agent of dermatophytosis, most likely representing the profile of the urban dermatophyte microbiota, followed by T. mentagrophytes, M. gypseum, T. tonsures, E. floccosum, and M. canis.^{29,10-12,14}

The anatomical site most commonly infected with dermatophytes was the nails, accounting for 62.26% of cases, which is consistent with studies in the literature. 1,27,9,15 The region of the foot ranked second as a site of involvement, accounting for 25.42% of cases, in agreement with previous studies. 1,2,9,15,16 However, some studies have reported the feet as the anatomical site most frequently affected by dermatophytosis. 3,10,11,17,18 Ranking third we found dermatophytosis of the body with 6,26% of cases, in accordance with the published literature, 1,2,3,10,17 followed by the inguinal region (3,42%), hand (1,65%), and scalp (0,99%).

In children aged ≤10 years, the most frequent site was the scalp, corroborating data from previous studies, with 22 (42.59%) cases of tinea capitis, 14 (63.6%) of them caused by M. gypseum, 6 (27.3%) caused by T. tonsurans, and 2 (9.1%) caused by M. canis. 1,10,17,19 Tinea capitis is a disease of childhood and its progression can vary from an acute to a chronic form. Therefore, understanding the epidemiology and ecology of tinea capitis in a particular country is

TABLE 3: Absolute and relative frequency of dermatophytes isolated by age group in 2426 positive cases

Dermatophyte species by age	0-1	0	11-2	.0		21-30		31-40	41-5	50	51-0	50	>60	
p = 0.0003	n	0/0	n	0/0	n	0/0	n	0/0	n	0/0	n	0/0	n	0/0
T. rubrum	27	51.92	110	95.65	124	98.41	300	98.68	565	95.76	658	97.63	549	97.17
T. mentagrophytes	2	3.85	2	1.74	1	0.79	4	1.32	23	3.90	15	2.23	10	1.77
M. gypseum	14	26.92	0	0.00	0	0.00	0	0.00	0	0.00	1	0.15	0	0.00
T. tonsurans	6	11.54	0	0.00	0	0.00	0	0.00	1	0.17	0	0.00	1	0.18
E. floccosum	0	0.00	1	0.87	1	0.79	0	0.00	1	0.17	0	0.00	4	0.71
M. canis	3	5.77	2	1.74	0	0.00	0	0.00	0	0.00	0	0.00	1	0.18
TOTAL	52	100	115	100	126	100	304	100	590	100	674	100	565	100

p< 0. 0001	0-1	.0	11-2	20		21-30		31-40	41-5	0	51-6	0	>60		TOT	AL
	n	0/0	n	0/0	n	0/0	n	0/0	n	0/0	n	0/0	n	0/0	n	0/0
Tinea unguium	11	20.37	40	38.10	66	49.25	188	63.30	384	63.74	448	65.98	374	67.63	1511	62.26
Tinea pedis	9	16.67	42	40.00	48	35.82	83	27.95	159	26.28	162	23.86	114	20.61	617	25.42
Tinea corporis	11	20.37	14	13.33	7	5.22	17	5.72	32	5.29	40	5.89	31	5.61	152	6.26
Tinea cruris	0	0.00	6	5.71	9	6.72	7	2.36	20	3.31	20	2.95	21	3.80	83	3.42
Tinea manuum	0	0.00	2	1.90	4	2.99	2	0.67	10	1.65	9	1.33	13	2.35	40	1.65
Tinea capitis	22	42.59	1	0.95	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	23	0.99
TOTAL	53	100	105	100	134	100	297	100	605	100	679	100	553	100	2426	100

TABLE 4: Absolute and relative frequency of dermatophytosis isolated by age group and subtype

TABLE 5: Frequency of dermatophytosis

	Total	
	N	0/0
Tinea unguium	1511	62.26
Tinea pedis	617	25.42
Tinea corporis	152	6.26
Tinea cruris	83	3.42
Tinea manuum	40	1.65
Tinea capitis	23	0.99
TOTAL	2426	100.00

important for educational and sanitary purposes and for the development of screening guidelines for detection of infectious foci and adoption of preventive measures.²⁰ Among individuals aged 11-20 years, tinea pedis was most frequent, accounting for 42 (40%) of cases.^{3,10} In all other age groups, tinea unguium was the most prevalent subtype.

The mean patient age in dermatophyte-positive cases was 49 years, and dermatophytosis was more frequently found in individuals over 40 years of age, with a female predominance (n=1860, 71%) consistent with previous studies. ^{1,4,10,19}

Pityriasis versicolor is a superficial mycosis caused by Malassezia that has a cosmopolitan distribution, occurring mainly in temperate and tropical climates. It is associated with several factors including poor health status, chronic infections, excessive sweating, and physiological states such as pregnancy, contraceptive use, and steroid therapy.²¹

In this study, most cases of Malassezia infection occurred in adulthood (mean patient age, 37 years). A possible explanation is that, because of the lipophilic nature of this yeast and the post-puberty hormonal stimulus inherent in this age group, there is stimulation of sebaceous glands accompanied by an increase in the fat content of the skin, which serves as a sub-

strate for the fungus. These results are in agreement with the literature. ²¹⁻²⁴ The most common sites were the chest and neck, with 141 (69%) cases, and other regions accounting for 64 (31%) of cases. This is probably due to the fact that these are the regions with the highest concentration of sebaceous glands, which is in agreement with data from previous studies. ^{21,22} Men and women are about equally affected. ^{22,23} In this study, 125 (61%) affected patients were female and 80 (39%) were male, which is also consistent with data from the literature reporting a variation in this prevalence, with some studies pointing toward male predominance. ^{24,25} and others toward female predominance.

Candida is a normal component of the human microbiota, being considered an opportunistic yeast that compromises, individually or together, mucous membranes, skin and nails. ^{1,6,7} As an agent of superficial mycoses, it has the ability to digest keratin present in the skin and its appendages, which may trigger an inflammatory host response. ¹ Among cases positive for Candida, the mean age of patients was 50 years, and 154 (81%) patients were female and 37 (19%) were male. The interdigital region and nails accounted for 171 (90%) of cases, and other regions for 20 (10%) of cases, which is consistent with the literature. ^{15,27,30}

CONCLUSION

In this study, the biota consisted of six dermatophyte species, T. rubrum, T. mentagrophytes, M. gypseum, T. tonsurans, E. floccosum, and M. canis, maintaining the trend of increased frequency of T. rubrum and stable values for T. mentagrophytes over time. The most common site of involvement was the nail and foot in adults and scalp in children, with a female predominance.

Both Candida and Malassezia were more prevalent in adult women, the former most commonly affecting the interdigital region and nails and the latter the chest and neck.

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