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FUNGAL AGENTS IN DIFFERENT ANATOMICAL SITES IN PUBLIC HEALTH SERVICES IN CUIABÁ, STATE OF MATO GROSSO, BRAZIL

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SUMMARY

Introduction: A contribution to the regional epidemiological profile of the most common fungal agents in Public Health Services in Cuiabá, state of Mato Grosso, including university hospitals and polyclinics. **Methods:** Clinical specimens (n = 1,496) from 1,078 patients were collected, submitted to direct mycological exam (potash or stick tape method) and cultured in specific mediums. Dermatophytic and non-dermatophytic agents were identified according to micromorphology (Ridell technique). **Results:** The majority of the 1,496 specimens were skin (n = 985) and nail exams (n = 472). Of the 800 positive cultures, 246 (30.8%) corresponded to dermatophytes and 336 (42%) to yeasts of the genus *Candida*, 190 (23.7%) to other yeasts, 27 (3.4%) to non-dermatophytic filamentous fungi and one (0.1%) the agent of subcutaneous mycosis. Lesions considered primary occurred in greater numbers (59.5%) than recurrent lesions (37.4%), with a greater concentration of positivity occurring on the arms and legs. **Conclusions:** Comorbidities, allergies and *diabetes mellitus* were conditions associated with greater positivity in direct mycological exams and cultures. Positive culture was considered a definitive diagnosis of fungal infection and confirmed 47.8% of diagnostic hypotheses.

KEYWORDS: Dermatomycoses; Fungal agents; Mato Grosso.

INTRODUCTION

In dermatology out-patient clinics, principally in tropical countries, cases of tineas (cutaneous/skin mycoses) and other superficial fungal infections presenting highly characteristic clinical aspects are observed daily, definitively favoring their diagnoses. Several factors affect the higher incidence of superficial and cutaneous mycoses, including: bioclimatic conditions favorable to the development of fungi in saprophytic life; promiscuity; sweating; prolonged contact with pets (cats and dogs), since they constitute potential reservoirs of certain dermatophytes; and contaminated water from swimming pools and surrounding risk areas (paving close to pools)[24].

Superficial and cutaneous mycoses can be detected on the skin, in hair, on the nails, in periungual folds, in the mucosa and cutaneomucosal zones. It is not possible to outline an exact profile of the epidemiology of superficial and cutaneous mycoses, because they are not diseases that require compulsory notification. Certain tineas are extremely contagious, provoking microepidemics in schools or micro-epizootics, the latter especially among captive animals (rabbits, guinea pigs, mice and rats), though they also occur in rural areas and occasionally do not provoke obvious clinical lesions[42,44,45].

Epidemiological studies in Brazil have demonstrated the distribution of etiological agents responsible for superficial and cutaneous fungal infections in several geographical regions. The literature contains data collected and analyzed from the states of São Paulo[9], Goiás[12], Rio Grande do Sul[3], Santa Catarina[47], Minas Gerais[30], Distrito Federal[8], Amazonas[16], Paraná[40] and Ceará[6].

However, our review found no record of any kind of study conducted in the state of Mato Grosso, despite the fact that the bioclimatic conditions are extremely favorable to the appearance of such fungal infections. Thus, this work aimed to contribute to current understanding regarding the etiological distribution of the most frequent fungal agents detected in the primary healthcare service of the Brazilian National Health System (Sistema Único de Saúde, SUS) in Cuiabá, MT, elucidating the demographic, clinical and laboratorial aspects in order to permit the institution of early, effective therapy.

MATERIAL AND METHODS

Casuistic: Between March 2006 and December 2010, 1,496 clinical specimens were collected from 1,078 patients with lesions located in different anatomical sites, who were attended to at the SUS polyclinics and university hospitals in Cuiabá, state of Mato Grosso. This study was approved by the Ethics in Research Committee of Hospital Universitário Julio Muller under protocol number 240/06 - CEP/HUJM.

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Demographic and clinical data and predisposing factors: Patient demographic data, including age, sex and place of residence, were registered in individual patient forms, together with clinical data regarding lesion time (months), category, lesion site and appearance, and predisposing factors, such as contact with animals, superficial traumas, comorbidities and prior use of medications, and later evaluated.

Clinical specimens examined: Samples of skin, hair, mucous and nails from patients with suspected dermatomycoses were collected by scraping the surfaces with sterile scalpel blades. From the scalp, cutaneous exfoliation and damaged hairs were removed with sterile tweezers. The materials collected were placed in previously labelled sterile petri-dishes.

Direct mycological examination: Direct mycological examination (DME) was performed using 20% KOH on skins flakes and 40% KOH on hair and nails to gently dissolve the clinical material in order to improve visualization of the fungal parasite. Exams performed by the adhesive-tape method were used on patients presenting non-flaking or minimally flaking lesions[23,24,49].

Culture isolation: Isolation of the primary fungus was performed simultaneously with direct microscopy in specific culture media: Sabouraud dextrose agar (DIFCO) with added chloramphenicol and Mycobiotic agar (DIFCO). The samples were incubated at 27 ºC for seven to 20 days and observed daily[23,24,49].

Use of chromogenic agar: Chromogenic CHROMagar medium (BBL) was only used to determine the purity of the isolated colonies suggestive of the genus Candida. To identify the yeast species, the classic methodology was used, including the following tests: germinative tube; zymogram, carbohydrate fermentation; auxanogram, carbohydrate assimilation; and the Ridell Technique, microculture[23,24,49].

Identification of mycelial fungi: Identification of the groups of dermatophytic and filamentous fungi was achieved by observation of the macro- and micro-morphological characteristics (the Ridell Technique) of the developed colonies and, in specific cases, by biochemical tests[23,24,49].

RESULTS

Of the 1,078 patients evaluated, 642 (59.6%) were women and 436 (40.4%) were men, with an age range from six months to 96 years old and a mean (standard deviation) of 38.8 (19.6) years-old. The majority of the patients (68%) were resident in the state of Mato Grosso and another 13.6% were resident in other states of the central-western region. Residents from the northern region contributed to the lowest proportion of cases (1.5%). An important portion of patients were concentrated in the age groups of 20 to 49 years old (53.2%) and 50 years old or over (32.4%). In contrast, the 1- to 9-year-old age group presented the lowest proportion of patients (3.8%).

For the mycological investigation, 1,496 clinical samples were collected, comprising skin flakes (65.8%), nail fragments (31.6%), mucous (1.8%), secretion (0.3%) and hairs (0.5%). The main cutaneous sites (n = 725) with suspected dermatomycosis lesions were the legs (21.2%), arms (16.1%) and thorax (15.8%). Among the phaneros (n = 428), the nails were by far the most commonly affected sites (98.1%) (Table 1). The disease period reported by the patients varied from one month to 40 years, with a median (Q1 to Q3) of 12 months (2 - 48 months). The majority of patients (48.1%) reported lesion times of more than six

Table 1
Clinical characteristics of the 1,078 patients with suspected fungal infections attended by the primary healthcare service of the Brazilian National Health Service (SUS), Cuiabá, MT, 2006-2010

Characteristic		n	%
Clinical specimen	Skin	725	61.2
	Nails	420	35.5
	Mucous	27	2.3
	Hair	8	0.7
	Secretion	4	0.3
Lesion site	**Skin**		
	Legs	154	21.2
	Arms	117	16.1
	Thorax	115	15.8
	Face	47	6.5
	Scalp	39	5.4
	Buttock	36	5.0
	Breast	7	1.0
	Ear	6	0.8
	Axilla	11	1.5
	Genital region	7	0.8
	Inguinal region	13	1.8
	Feet	89	12.3
	Hands	36	5.0
	Other regions	50	6.8
	Phaneros		
	Nails	420	98.1
	Hair	8	1.9
Lesion time (months)	< 1	118	10.0
	1 –| 3	265	22.4
	3 –| 6	189	16.0
	> 6	570	48.1
	not reported	42	3.5
Lesion clinical aspect*	Cutaneous exfoliation	451	38.1
	Opaque/brittle nail	384	32.4
	Cutaneous ulcer	73	6.2
	Cutaneous scab	61	5.2
	Cutaneous hypochromia	73	6.2
	Cutaneous blister	19	1.6
	Cutaneous nodule	6	0.5
	Not reported	117	9.9
Lesion evolutive category	Primary	705	59.5
	Recurrent	443	37.4
	Not reported	36	3.0

*: exfoliation, itching and erythema were present in the lesions of clinical specimens 1093, 830 and 641, respectively.

months duration. The types of lesions were presented in the following proportions: cutaneous exfoliation (38.1%), opaque/brittle nails (32.4%), cutaneous ulcerations (6.2%) and cutaneous scabs (5.2%). The triad that characterizes dermatophytoses; i.e. flaking, itching and erythema, were observed in the majority of lesions and were present in lesions 1093, 830 and 641, respectively. Primary lesions predominated (59.5%), with only 37.4% of patients presenting recurrent lesions (Table 1).

Table 2 presents the likely predisposing factors for fungal infection in suspected lesions observed in patients attended by the primary healthcare service of the SUS, Cuiabá, MT, in the period from 2006 to 2010. *Diabetes mellitus* (6.0%) and allergies (3.7%) were the most frequent comorbidities reported by the patients in the study. Dogs were the most commonly reported pet / domestic animal (42.5%), followed by cats (14.3%). Prior history of superficial trauma was denied by the majority of patients (90.4%).

Table 3 presents the frequency of fungal genera and species identified in superficial, cutaneous and subcutaneous lesions of patients attended by the primary healthcare service of the SUS, Cuiabá, MT, in the period from 2006 to 2010. Observation verified the predominance of yeasts

Table 2
Probable predisposing factors for 1,078 fungal infections in suspected lesions attended to by the primary healthcare service of the Brazilian National Health Service (SUS), Cuiabá, MT, 2006-2010

Factor		n	%
Contact with animals	Dogs	458	42.5
	Cats	154	14.3
	Pet birds	32	3.0
	Other domestic animals	30	2.8
	Other sources of contamination	404	37.5
Previously reported trauma	Contusions	30	2.8
	Punctures	14	1.3
	Abrasions	16	1.5
	Surgery	5	0.5
	Cuts	14	1.3
	Prolonged pressure	13	1.2
	Others	12	1.1
	None	974	90.4
Prior use of medications	Antifungal drugs	136	12.6
	Corticosteroids	48	4.5
	Antibiotics	38	3.5
	Home-made remedies	7	0.6
	Others	156	14.5
	None	693	64.3
Reported comorbidity	*Diabetes mellitus*	65	6.0
	Allergy	40	3.7
	AIDS	23	2.1
	Lupus erythematosus	11	1.0
	Hypothyroidism	4	0.4
	Others	85	7.9
	None	850	78.8

of the genus *Malassezia* 176 (22%) in skin lesions, indicating a high frequency of cases of pityriasis versicolor in the population evaluated. In relation to hair clinical material, only five species were isolated: two cases of *Trichophyton tonsurans, Trichosporon cutaneum* and other *Microsporum canis*. We performed 16 collections relating to mucous and secretion, resulting in isolation of *Candida albicans* 15 (93.8%) and *Candida parapsilosis* one (6.3%). In skin lesions, *T. rubrum* corresponded to 101 (20.3%) of the isolates and *T. mentagrophytes* to 37 (7.4%); these constituted the majority of the dermatophytes identified.

DISCUSSION

A series of studies conducted in Brazil since the 1960s indicates the distribution of causative agents of superficial mycoses and dermatophytic agents in numerous cities and Brazilian regions[3,6,8,10,12-14,19,26,27,30,32,39,40,43,46-48,51,54]. The reports of fungal infections and their agents in populations in world, have been addressed by several authors[2,15,21,22,29,31,52,58].

Dermatophytes are known to grow best in warm and humid environments and are, therefore, more common in tropical and subtropical regions. Some species for dermatophytes such as *Trichophyton mentagrophytes* var. *interdigitale, Microsporum canis, Epidermophyton floccosum* and *Trichophyton rubrum* are relatively common in several regions of the globe. Other species have partial geographic restriction; for example, *Trichophyton violaceum* is found in Asia, Africa and Europe; *Trichophyton concentricum* in the India and the Pacifics[2] and *Trichophyton schoenleinii* and *Trichophyton soudanense* is restricted to Africa[53]. In this study the species reported predominantly in the central western part of Brazil that are distributed all over the world were: *Trichophyton rubrum, Trichophyton mentagrophytes; Trichophyton verrucosum, Trichophyton tonsurans, Microsporum canis* and *Microsporum gypseum*. Among the yeasts, we can highlight *Candida albicans*, *Candida parapsilosis* and *Malassezia* spp., which have also been isolated as agents of superficial and cutaneous mycoses.

In recent times, infections caused by dermatophytes have assumed greater significance. The increasing number of patients with immunocompromised states, such as AIDS, *diabetes mellitus,* lupus, allergy, cancer and organ transplantation, has given these infections more prominence[5,18,20,28,37,38,56]. Reports of infections by non-dermatophytes has also received greater attention[4,7,34,51]. In this study, *Diabetes mellitus* (6.0%) and allergies (3.7%) were observed in patients. These were the most frequent comorbidities reported by the patients.

Regarding skin and nail dermatophytes, mycotic lesions are less frequent among children, who present fewer keratinized sites than adults[41]. Onychomycosis is more common in diabetic patients than in non-diabetic patients. It is more than a cosmetic problem, and diabetic patients have a greater risk of serious complications with the disease[55]. The predominance of women among individuals with superficial and cutaneous fungal infections has been reported by other authors[4,14,34,36,57], who proposed the hypothesis that clinical manifestations of certain dermatomycoses (e.g. in nails) could be predominantly cosmetic and affect women more directly than men.

Among the group of superficial and cutaneous fungal infections, candidiasis should be highlighted. Onychomycosis of fingernails is most

Table 3
Frequency of fungal genera and species (n=800) identified and diagnosed in patients attended by the primary healthcare service of the Brazilian National Health Service (SUS), Cuiabá, MT, 2006-2010.

Clinical specimen	Genus/species	n	%
SKIN (n = 498)	*Malassezia* spp.	176	35.3
	Trichophyton rubrum	101	20.3
	Candida parapsilosis	77	15.5
	Trichophyton mentagrophytes	37	7.4
	Candida albicans	22	4.4
	Microsporum canis	16	3.2
	Candida tropicalis	15	3.0
	Epidermophyton floccosum	12	2.4
	Microsporum gypseum	8	1.6
	Trichosporon spp.	5	1.0
	Candida guilliermondii	4	0.8
	Trichophyton tonsurans	3	0.6
	Trichophyton violaceum	3	0.6
	Candida famata	3	0.6
	Candida kefyr	3	0.6
	Candida lusitaniae	2	0.4
	Candida lipolytica	2	0.4
	Trichophyton verrucosum	2	0.4
	Candida glabrata	2	0.4
	Candida sp.	1	0.2
	Exophiala werneckii	1	0.2
	Trichophyton interdigitali	1	0.2
	Sporothrix schenckii	1	0.2
	Candida rugosa	1	0.2
TOENAILS (n = 166)	*Candida parapsilosis*	65	39.2
	Trichophyton rubrum	28	16.9
	Trichophyton mentagrophytes	14	8.4
	Fusarium spp.	16	9.6
	Candida albicans	10	6.0
	Scopulariopsis brevicaulis	6	3.6
	Candida tropicalis	6	3.6
	Trichosporon spp.	4	2.4
TOENAILS (n = 166)	*Candida kefyr*	3	1.8
	Trichophyton tonsurans	2	1.2
	Scytalidium dimidiatum	2	1.2
	Candida guilliermondii	2	1.2
	Trichophyton verrucosum	2	1.2
	Candida famata	1	0.6
	Candida rugosa	1	0.6
	Candida lusitaniae	1	0.6
	Trichophyton interdigitali	1	0.6
	Candida glabrata	1	0.6
	Candida krusei	1	0.6
FINGERNAILS (n = 112)	*Candida parapsilosis*	53	47.3
	Candida albicans	16	14.3
	Candida tropicalis	15	13.4
	Trichophyton rubrum	7	6.3
	Trichophyton mentagrophytes	6	5.4
	Candida glabrata	3	2.7
	Fusarium spp.	3	2.7
	Candida guilliermondii	2	1.8
	Candida lusitaniae	2	1.8
	Trichosporon spp.	2	1.8
	Candida famata	1	0.9
	Candida krusei	1	0.9
	Candida viswanathii	1	0.9
HAIR (n = 5)	*Trichophyton tonsurans*	2	40.0
	Trichosporon cutaneum	2	40.0
	Microsporum canis	1	20.0
MUCOUS (n = 16)	*Candida albicans*	15	93.8
	Candida parapsilosis	1	6.2
SECRETION (n = 3)	*Candida albicans*	2	66.7
	Candida parapsilosis	1	33.3

prevalent in females while toenail infection is common in male patients[1,57]. *Candida* species is the dominant cause of onychomycosis in women and dermatophytes is the principal cause of this condition in men[35,51]. According to CROCCO *et al.*[13], candidiasis expresses the variety of relations that occur between the host and autochthon microbiota, ranging from commensalism to systemic disease. Different groups of previously used medications can contribute to the appearance of superficial and systemic fungal infections by yeasts of the genus Candida[11]. In this study, antifungal drugs were reported by 12.6% of patients, followed by corticosteroids (4.5%) and antibiotics (3.5%).

Causative agents of dermatomycoses were isolated from 47.8% of the samples cultured for fungi. These cultures were interpreted as proof of the diagnostic hypothesis of mycosis. Verification of 47.8% of positive cultures is in agreement with the range of positivity that has been reported in the literature for these types of mycoses, which vary between 20.8% and 60%, when culture is considered a diagnostic criterion[40,50]. Several causes have been indicated to justify the low rate of fungi recovery in culture media, as registered in this study. Among these, two should be highlighted: prior use of medication, a factor frequently underreported by patients; and equivocal clinical diagnosis, related to the existence of numerous skin lesions and attachments that are clinically similar to mycoses, though with distinct etiologies[33].

In the state of Paraná, six cases of *Tinea nigra* were reported in 2003, corresponding to cases diagnosed between 1978 and 2001[17]. This small

casuistic corroborates the infrequent character or subdiagnosis of this fungal infection. In toenail lesions, the following species were the most frequent: 28 isolates of *T. rubrum* (16.9%) and 14 of *T. mentagrophytes* (8.4%), contrasting with the lower rate of isolation in fingernails, seven isolates of *T. rubrum* (6.3%) and six of *T. mentagrophytes* (5.4%).

Regarding nail lesions (fingers and toes), it can be inferred that the extreme adaptation achieved by these microorganisms in relation to the human host, together with the chronicity so frequently associated with lesions involving these sites, permit the novel hypothesis that the low rate of culture positivity could be due to the inability to grow *in vitro* microorganisms so well adapted to human tissue for such a long period of time. LEYDEN (1994)[25] further suggests, specifically regarding *Tinea pedis*, that the presence of maceration implies the establishment of secondary bacterial infection.

In conclusion, yeasts of the genus Candida predominate dermatophytes as agents of dermatomycoses at all the sites evaluated, except on hairs. The high frequency of these yeasts on nails can be attributed to the significant isolation of *Candida parapsilosis*, which is a component of the microbiota of this anatomic site. Among the dermatophytes, *T. rubrum* and *T. mentagrophytes* were the most frequently identified agents of suspected dermatomycosis lesions. Finally, it should be highlighted that resistance to antifungal drugs is growing and is one of the reasons that characterization of the etiological agent at the level of genus and species has been indicated as an important support for the choice of therapeutic management.

RESUMO

Agentes fúngicos em diferentes sítios anatômicos nos Serviços de Saúde Pública em Cuiabá, Mato Grosso, Brasil

Introdução: Contribuição sobre o perfil epidemiológico regional referente aos agentes fúngicos mais freqüentes nos Serviços de Saúde Pública em Cuiabá-MT, incluindo policlínicas e hospitais universitários. **Métodos:** Foram examinados 1.496 espécimes clínicos colhidos a partir de 1.078 pacientes, os quais foram submetidos ao exame direto (potassa e/ou fita gomada) e cultivos em meios específicos. Os agentes foram identificados segundo micromorfologia (técnica de Ridell). **Resultados:** Os 1.496 espécimes foram relacionados na maioria a exames de pele (n = 985), e unhas (n = 472). Dos 800 cultivos positivos, 246 (30,8%) corresponderam a dermatófitos, 336 (42%) a leveduras do gênero *Candida*, 190 (23,7%) a outras leveduras, 27 (3,4%) a fungos filamentosos não dermatofíticos e um (0,1%) a agente de micoses subcutâneas. Lesões consideradas primárias compareceram em maior número (59,5%), comparadas as recidivantes (37,4%). Foi observada maior positividade em membros inferiores e superiores. **Conclusões:** Co-morbidades, quadros alérgicos e *diabetes mellitus* representaram condições associadas à maior positividade em exames micológicos diretos e cultivos. O cultivo positivo foi considerado como diagnóstico definitivo de infecção fúngica, e confirmou 47,8% de hipóteses diagnósticas.

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