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Children's public health: Danger of exposure to pathogenic fungi in recreational places in the middle-west region of Brazil

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ABSTRACT

Background: Dermatophytes are keratinophilic fungi, considered etiological agents of cutaneous mycoses in man and animals. The objective of this work was to isolate dermatophytic and non-dermatophytic fungi in recreational sandboxes (rainy and dry periods) in public day-care centers located in the city of Cuiabá-MT (Middle-west of Brazil).

Methods: Samples (n = 200) were collected from the superficial layer of the sandboxes at a depth of 2–5 cm in 10 public nurseries in Cuiabá-MT. Hair baits measuring 1–2 cm were autoclaved and dispersed in 50 g of the sample in sterile plates, incubated at 28 °C, moistened with chloramphenicol solution (50 mg/L). After 6 weeks the baits were inoculated on Sabouraud agar plus 50 mg/L chloramphenicol and 500 mg/L cycloheximide. The fungi were identified according to macroscopic and micromorphological characteristics.

Results: From the collected sand samples, 1318 colonies and 56 species of fungi belonging to 22 genera were isolated. The most important genera were *Paecilomyces* spp. (30.42%), *Penicillium* spp. (19.12%), *Fusarium* spp. (11.46%) and *Aspergillus* spp. (11.15%). Dermatophytes were recovered in 50% of day-care centers in a total of 29 identified colonies, the *Trichophyton* genus (86.2%) being the most frequently isolated.

Conclusions: The dermatophytes in the recreational areas of day-care centers can pose a risk to the health of its users, especially for children and the employees. Work with this profile is very important to guide actions related to health surveillance.

Introduction

Fungi play an important role in nature; when restricted in this ecological niche, they assume responsibility for the natural decomposition of dead vegetal and animal organisms [1]. They develop from adequate nutrition and a favorable environment of humidity and temperature; they reproduce asexually and/or sexually according to the species and their life cycle [2]. Dermatophytes are a group of fungi that comprise nine genera: *Trichophyton* (*T.*), *Epidermophyton* (*E.*), *Nannizzia* (*N.*), *Microsporum* (*M.*), *Lophophyton* (*L.*), *Arthroderma* (*A.*), *Ctenomyces* (*C.*), *Guarromyces* (*G.*) and *Paraphyton* (*P.*),which represent keratinophilic fungi with special ability to invade the more superficial keratinized tissues of man and other animals causing dermatophytoses, characterized by the growth of microorganisms on or in hairs, in the stratum corneum of the epidermis in the hair follicles, nails for humans and claws, fur, horns, hooves for animals [3,4]. The species of this group were divided into three categories according to their natural habitats: anthropophilic, zoophilic and geophilic species [2].

Ecologically speaking, information on the relative role of each species in the degradation of keratin in nature leads to an understanding of the pathogenicity of species in humans and other animals [5]. The soil presents the main environment for the distribution of dermatophytes and other keratinophilic fungi, being the object of study of this work (sand), which is poor in organic and vegetal matter. It is worth noting that sand becomes enriched by fragments of skin, feathers, faeces, fur and claws due to high

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concentrations of people and domestic animals and synanthropes [6].

Genera and species of keratinophilic fungi found in soils from all parts of the world have been reported since the 1960s [7] and the distribution of these fungi in public parks and recreation areas has been registered in several countries such as Italy [5], Iran [8,9], France [10], Poland [11,12], India [13], Argentina [14] and Brazil [15–17]. One of the few works registered in Brazil and most especially in the Middle West [18], was carried out including the state of Mato Grosso, but it was based on a reduced number of samples. The availability of organic tissues (faeces, hair, feathers, fur and claws) favors the colonization of pathogenic fungi in recreational areas, which may represent a risk for opportunistic infections.

In addition to dermatophyte fungi, other groups of mycelial fungi that are present in the soil can also cause superficial or systemic infections. Considering their ability to cause injury, fungi are classified into three levels of biosafety (BSL) for humans. BSL 1 comprises saprophytes and plant pathogens, which can cause mild infections on rare occasions. The BSL 2 group includes pathogens responsible for infections in patients with immune system disorders. And pathogens in the last group, BSL 3, are capable of causing severe systemic infections in healthy individuals. Mycoses caused by mycelial fungi usually affect people with compromised immune systems [19].

Among the non-dermatophytic filamentous fungi, it is worth mentioning the genus Fusarium as a potential cause of hyalohyphomycosis involving lower limbs, genitourinary tract, nasal cavity, lung, gastrointestinal tract, eyes and skin. Penicillium genus may be the etiological agent of respiratory, gastrointestinal and skin infections [20]; Acremonium genus have also been isolated from clinical materials such as sputum, bronchoalveolar lavage, eyes and nails [21,22].

The objective of this study was to isolate and identify dermatophytic and non-dermatophytic fungi in sand parks of public day-care centers in Cuiabá – Mato Grosso, emphasizing on species that are potentially pathogenic to all users.

Materials and methods

Study scope

Samples were collected in public day-care centers in the city of Cuiabá – MT (15° 35′45″S, 56° 5′ 49″ W), Middle West of Brazil, capital of the state of Mato Grosso, located at 151 m above sea level [23]. Its climate is tropical and humid, it is located in one of the hottest regions of Brazil. The annual average relative humidity is 80%, the average monthly temperature is approximately 27 °C in the months of October to March, however it frequently reaches temperatures of 40 °C. Its average rainfall is 1400 mm per year [24].

The sampling sites are located in the urban area of the city, 3 of them in the Northern area, 3 in the South, 3 in the East and 1 in the Western region. A total of 56 nurseries were surveyed for the presence of sandboxes, but only 29 were localized [25].

Sand samples

The samples were collected in areas close to the playground toys, arranged in the places most used by the users. Sampling was carried out at ten points equidistant from each of the sand boxes, at 10 municipal nurseries, and a total of 200 samples were obtained. One hundred samples were obtained between February 20 and March 21, 2017 (rainy season) and 100 samples from June 29 to July 10, 2017 (dry season). The samples were collected through the availability of the day care team, which was performed in a single day in each unit. The material was obtained with the aid of sterile spatula, taking it just below the surface of the soil (2–5 cm) and collected in pre-sterile falcon tubes. Each sample consisted of 100 g (±5) sand, which were enveloped in plastic film.

Fungal culture, isolation and identification of fungi

The hair baiting technique of Vanbreuseghem [8,9,26] was used for the culture and isolation of keratinophile filamentous fungi. Each sand sample was thoroughly homogenized, and approximately 50 g of sand were packed into a 90 mm sterile petri dish. Then, several pieces of sterile and healthy hair fragments were dispersed on the surface of the sand samples and moistened with sterile distilled water supplemented with antibiotic solution (chloramphenicol, 50 mg/L).

All Petri dishes were incubated at room temperature (28 °C) in B.O.D. (Biochemical Oxygen Demand) incubators for 6 weeks and were moistened when necessary. After observing the growth of the colonies around the baits, the colonies were subcultured on Sabouraud dextrose agar (SDA) with chloramphenicol (50 mg/L) and cycloheximide (500 mg/L) and purified [2,9,27]. Cycloheximide acts in the complete or partial inhibition of the growth of non-dermatophytic mycelial fungi which are usually grown faster in vitro, thus allowing the isolation of the dermatophytic fungi.

The genera and species of the dermatophyte and non-dermatophyte fungi were identified based on the conventional method (colony morphology and macro and microconidia characteristics), using microculture technique and standardized identification techniques [2,27,29]. All fungal isolates were preserved using the Castellani technique [30], at the Mycology/Investigation Laboratory, Medicine School – Federal University of Mato Grosso/Cuiabá – Mato Grosso, Brazil.

A 1:5 suspension of distilled water (m/v = one gram of sand to five ml of distilled water) was used to measure the pH of the sample on a pH meter (MS TECNOPON – Instrumentation, mPA210) [28].

Statistical analysis

The categorical variables were summarized by means of absolute and relative frequencies. Numerical variables were summarized by means of medians and interquartile ranges (IQR) or means and 95% confidence intervals (95% CI) after using Shapiro–Wilk test to verify their normality.

To evaluate the correlation between two numerical variables, the Pearson correlation coefficient or its analogue Spearman coefficient was used. To compare the number of isolates between the first season (rainy season) and the second period (dry season) the paired Student's *t*-test or its nonparametric analogue Wilcoxon paired test was used. To compare the number of isolates per region, the ANOVA or its non-parametric analogue Kruskal–Wallis test was used. A significant value of $p < 0.05$ was considered.

Results

Out of the total nurseries evaluated (n = 10), dermatophytic fungi were isolated in 50.0% of them in the rainy season and in 20.0% in the dry season. Altogether, half of the day-care centers had dermatophyte isolation in both periods.

In all, 1121 children attend these kindergartens and are looked after by 413 employees. Table 1 shows the percentage variation of the number of colony forming units of dermatophytic and non-dermatophytic fungi, for each of the nurseries between the rainy and dry periods. The mean number of colony forming units was statistically higher in the dry period, a difference adjusted by the isolates profile of day-care centers in the eastern area of Cuiabá/MT.

Table 2 shows that there were no differences between the groups of dermatophytes and non-dermatophytes compared in the

Table 1
Number of general colony forming units isolated from the soil of Cuiabá/MT day-care in 2017, according to the seasonality.

Regions	Period		Variation (%)
	Rainy	Dry	
North			
Day-care 1	34	99	191.2
Day-care 2	69	82	18.8
Day-care 3	46	86	86.9
Average (IC95%)	50 (5–93)	89 (67–111)	
South			
Day-care 4	37	84	127.0
Day-care 5	25	97	288.0
Day-care 6	67	75	11.9
Average (IC95%)	43 (1–97)	85 (58–113)	
East			
Day-care 7	38	77	102.6
Day-care 8	43	71	65.1
Day-care 9	46	89	93.5
Average (IC95%)	42 (32–52)	79 (56–102)*	
West			
Day-care 10	77	76	−1.3
General average (IC95%)	48 (36–60)	84 (77–90)*	

* $p < 0,05$ for the inter-period test.

seasonal periods. However, when an inter-group comparison was performed, there was a significant variation between dry and rainy periods, statistically confirmed by $p < 0.05$, affirming that the highest percentage of CFUs recovered by dermatophytes occurred in the rainy season (82.8%), and for non-dermatophytes the highest percentage occurred in the dry period (64%) CFU's.

From the 200 collected samples, 1318 colonies of filamentous fungi were isolated and later identified, corresponding to the 56 species of fungi belonging to 22 genera. Table 4 shows the isolates recovered from all day-care centers.

The most frequently recovered were fungi of the genera; *Paecilomyces* (30.42%), *Penicillium* (19.12%), *Fusarium* (11.46%), *Aspergillus* (11.15%) from 10 day-care centers, *Mycelia* (6.3%), *Acremonium* (5.16%), *Cladosporium* (5.01%) in 7 day-car centers, followed by other isolated genera in lower percentages. Among the 22 genera of identified fungi, 17 were recovered in both periods, and 5 were recovered only in the rainy season, namely *Cunninghamella* (0.83%), *Microsporum* (0.83%), *Mucor* (0.41%), *Phialophora* (0.21%) and *Sepedonium* (0.41%). In the dry period, the following genera were isolated: *Cladosporium* (7.89%), *Epicoccum* (0.72%), *Monascus* (0.24%), *Scedosporium* (0.72%) and *Tritirachium* (0.48%) (Table 2).

The correlation analysis showed that day-care centers with positive dermatophyte isolates had presented results of soil pH, relative humidity, the total number of children in each day-care center and the total number of employees working there, statistically similar to day-care centers where there were no dermatophytes isolates (Table 3).

However, when the dry period was evaluated, there was a statistically strong, negative and significant correlation between the number of colonies forming units of dermatophytes and the relative humidity of the same period ($rho = -0.64$, $p < 0.05$).

That is, the higher the value of the relative humidity of the air, the lower the number of dermatophyte isolates for this period. These differences were not observed in the rainy season.

Discussion

Due to their role in the degradation of keratinized waste, keratinophilic fungi have attracted the attention of many researchers, and, within this scope, many studies have been carried out worldwide aiming to isolate this group of microorganisms from different types of soil with emphasis on recreational parks.

The importance of these studies is obviously directly related with the epidemiological chain linking these residues, animals that may be frequently present in these places, and children who usually spend time in recreational activities, and can thus acquire dermatophytoses. These infections are cosmopolitan, being distributed all over the world, in countries with different climates, relative humidity, soil types regarding organic compounds, pH, and chemical compounds.

Several factors condition the higher incidence of dermatophytoses, such as bioclimatic conditions which can be favorable to the development of fungi in saprophytic life. Among these factors, the confinement of populations (children and adults), prolonged contact with small animals whether domestic (such as cats and dogs) or wild, contaminated water in swimming pools and reservoirs, are some situations that lead to the appearance and proliferation of fungi. One other important aspect to note is the abusive use of immunosuppressants, mainly corticosteroids in the last decades, which led to the appearance of generalized dermatophytosis involving cutaneous infections. In addition, the immature immune system of children in the evaluated age group in this study (0–5 years) must also be considered [9,12,27].

The distribution of dermatophytoses and their etiological agents also varies according to the geographic region and socioeconomic status of the population, in this case considered cosmopolitan, but regional distributions are observed when exposed to risk situations [2,4].

Generally speaking, it is possible to observe that several groups of researchers have registered distinct percentages referring not only to dermatophytes found in these places, but also non-dermatophytic filamentous fungi. The results obtained vary directly related to the different seasons in which they are recovered (spring, autumn), or climatic periods (rainy or dry), geological constitution of the soil and variations in its pH, and environmental factors among others [12,31–34].

Considering the two groups of fungi isolated in this work, the dermatophytic fungi will be approached with greater emphasis due to their importance as classic agents of dermatophytoses.

The risk of acquiring dermatophytoses is significant for children, who represent the target public susceptible to these etiological agents due to their constant exposure in school parks, day-care centers or even public squares [16].

For example, *M. gypseum* has been universally isolated from acid soils [35], *T. terrestre* has been preferentially found in alkaline or neutral soils [36], while *T. ajelloi* has been isolated in acidic soils [26], neutral soils [36] and alkaline soils [37]. Several studies [15,28,17] have shown that the distribution of dermatophytes and

Table 2
Comparison of means and their respective 95% confidence intervals of fungal isolates from the soil of Cuiabá/MT day-care in 2017, according to the seasonality.

Period	Fungal Group		Comparison intergroup p-value
	Dermatophytes	Non-dermatophytess	
Rainy	46.4 (22.6–70.2)	50.0 (29.2–70.8)	0.76
Dry	83.0 (6.8–159.2)	83.7 (75.3–92.2)	0.92
Comparison intragroup/p-value	0.03	0.04	

Table 3
Frequency of distribution of dermatophyte and non-dermatophyte fungi according to gender and species isolated in sandboxes in Cuiabá day-care centers, according to seasonality, Feb–Mar and Jun–Jul 2017.

Genus–species	Rainy season		Dry season		Total	
	CFU's[a]	%	CFU's	%	CFU's	%
Dermatophytess						
Trichophyton rubrum	6	25.0	3	60.0	9	31.1
Trichophyton mentagrophytes	6	25.0	0	0.0	6	20.7
Trichophyton tonsurans	5	20.8	0	0.0	5	17.2
Trichophyton verrucosum	3	12.5	2	40.0	5	17.2
Microsporum gypseum	4	16.7	0	0.0	4	13.8
Total dermatophytes	24	100.0	5	100.0	29	100.0
Non-dermatophytess						
Paecilomyces lilacinus	132	28.8	223	26.8	355	27.5
Penicillium citrinum	32	7.0	89	10.7	121	9.4
Fusarium solani	20	4.4	69	8.3	89	6.9
Mycelia sterilia	27	5.9	56	6.7	83	6.4
Cladosporium spp.	0	0.0	65	7.8	65	5.0
Penicillium glabrum	30	6.6	27	3.2	57	4.4
Acremonium killiense	4	0.9	45	5.4	49	3.8
Penicillium chrysogenum	42	9.2	3	0.4	45	3.5
Chrysosporium spp.	26	5.7	8	1.0	34	2.6
Paecilomyces viridis	4	0.9	28	3.4	32	2.5
Aspergillus terreus	4	0.9	24	2.9	28	2.2
Aspergillus flavus	7	1.5	18	2.2	25	1.9
Aspergillus japonico	0	0.0	25	3.0	25	1.9
Aspergillus parasiticus	3	0.7	20	2.4	23	1.8
Fusarium spp.	12	2.6	11	1.3	23	1.8
Acremonium spp.	19	4.1	0	0.0	19	1.5
Aspergillus fumigatus	6	1.3	12	1.4	18	1.4
Aspergillus niger	6	1.3	9	1.1	15	1.2
Chrysosporium fastidium	0	0.0	14	1.7	14	1.1
Fusarium chlamydosporum	5	1.1	8	1.0	13	1.0
Fusarium proliferatum	4	0.9	9	1.1	13	1.0
Talaromyces funiculosus	13	2.8	0	0.0	13	1.0
Aspergillus carbonario	0	0.0	12	1.4	12	0.9
Penicillium spp.	9	2.0	0	0.0	9	0.7
Penicillium variotii	9	2.0	0	0.0	9	0.7
Paecilomyces variotii	3	0.7	5	0.6	8	0.6
Rhyzopus oryzae	2	0.4	6	0.7	8	0.6
Graphium/pseudallescheria boydii	7	1.5	0	0.0	7	0.5
Trichoderma viride	4	0.9	3	0.4	7	0.5
Epicoccum nigrum	0	0.0	6	0.7	6	0.5
Paecilomyces spp.	0	0.0	6	0.7	6	0.5
Scedosporium apiospermum	0	0.0	6	0.7	6	0.5
Fusarium oxysporum	2	0.4	3	0.4	5	0.4
Talaromyces variabilis	0	0.0	5	0.6	5	0.4
Cunninghamella bertholletiae	4	0.9	0	0.0	4	0.3
Fusarium dimerum	4	0.9	0	0.0	4	0.3
Fusarium graminearum	4	0.9	0	0.0	4	.0.3
Penicillium corylophilum	4	0.9	0	0.0	4	0.3
Tritirachium oryzae	0	0.0	4	0.5	4	0.3
Graphium spp.	0	0.0	3	0.4	3	0.2
Penicillium purpurascens	0	0.0	3	0.4	3	0.2
Trichoderma spp.	3	0.7	0	0.0	3	0.2
Monascus ruber	0	0.0	2	0.2	2	0.2
Penicillium aurantiogriseum	0	0.0	2	0.2	2	0.2
Penicillium griseofulvum	2	0.4	0	0.0	2	0.2
Sepedonium spp.	2	0.4	0	0.0	2	0.2
Aspergillus ochraceus	0	0.0	1	0.1	1	0.1
Cladosporium cladosporioide	0	0.0	1	0.1	1	0.1
Mucor circinelloides	1	0.2	0	0.0	1	0.1
Mucor sp.	1	0.2	0	0.0	1	0.1
Phialophora parasitica	1	0.2	0	0.0	1	0.1
Total non-dermatophytes	458	100.0	831	100.0	1289	100.0
Total geral	482		836		1318	

[a] CFU's — colony forming units.

other keratinophilic fungi in the soil is influenced by the enrichment produced by organic materials, particularly by skin scales, hair, feces and others. For this reason, organic residues probably contaminate the soil directly with fungal propagules and these can infect several anatomical sites in humans [5].

Most species that cause dermatophytosis have become adapted to people or animals, and are now maintained in these reservoirs. Although they can infect other hosts, each dermatophyte tends to be associated with a particular host or group of hosts, and it is not maintained in other species long term. It was a species complex containing many species and varieties with different morphological characters in the past.

Zoophilic members of Trichophyton include: *T. verrucosum*, *T. bullosum* and *T. mentagrophytes* complex. Most or all zoophilic

Table 4
Comparison of quantitative variables according to the presence of dermatophytes isolated in day care centers of Cuiabá/MT in 2017.

Variable	Isolation of dermatophytes in day care		P
	Yes	No	
Rainy season			
pH[a]	8.3 (7.7–8.9)	8.7 (7.5–9.9)	0.47
Number of CFU's[b] isolated	46 (22–70)	50 (29–70)	0.76
Relative humidity (%)	75.6 (56.4–94.8)	72.0 (51.3–92.7)	0.73
Dry season			
pH	8.7 (7.8–9.5)	8.4 (7.0–9.8)	0.63
Number of CFU's isolated	85 (74–96)	82 (69–95)	0.66
Relative humidity (%)	63.0 (43.3–82.7)	64.6 (46.7–82.5)	0.87
Number of children	104 (69–139)	120 (41–200)	0.62
Number of employees	40 (30–49)	43 (17–69)	0.74

[a] pH — hydrogenation potential.
[b] CFU's — colony forming units.

dermatophytes are thought to be zoonotic, although some are transferred to people more often than others. *T. verrucosum* and *T. equinum* are the species usually isolated from ruminants and horses, while *T. mentagrophytes*, which affects a range of different rodent species or, rarely, cats, dogs, and horses [3,38].

In 1999 in India (Madras), keratinophilic fungi were recovered from the soil of 30 primary schools and 15 public parks, isolating 31 species belonging to 15 genera. Regarding dermatophytes, four species (*M. gypseum*, *M. nanum*, *T. mentagrophytes* and *T. terrestre*) were isolated, out of which *M. gypseum* was the most frequently isolated species (48.8%) in schools and parks followed by *T. mentagrophytes* (37.7%), *T. terrestre* (31.1%), while *M. nanum* was recovered in 10% of the evaluated schools [13].

Comparing the results obtained by Ramesh & Hilda [13] with those obtained in this study, it is possible to verify that only *M. gypseum* was isolated from day-care centers in Cuiabá, Brazil, considering the genus Microsporum, in contrast with the genus Trichophyton, with the isolation of four species, namely: *T. mentagrophytes*, *T. rubrum*, *T. tonsurans* and *T. verrucosum*, with percentages already shown in the results section.

It is important to mention that many species found in these soils, and responsible for the degradation of keratin, are considered as etiological agents of fungal infections, both in animals and in humans [16].

T. mentagrophytes complex is known for its polymorphic character and may appear in either zoophilic, anthropophilic and geophilic state, but it is always zoophilic [13]. For this reason, it becomes an important species considering the epidemiological chain, considering that this species was recovered from the samples from day-care centers in Cuiabá, Brazil.

Another interesting work reported the different species of dermatophytes isolated from soil in Jordan, considering the different types of terrain: valley, mountain and desert soils. Ten samples were obtained from each different type of soil associated with the terrain; out of these 30 samples, 15 samples were positive for dermatophytes, 9 of which came from mountain soils, six from valleys and nine from desert soils. A total of 42 isolates were identified, 24 belonging to the genus *Microsporum* and 18 to the genus *Trichophyton*. Among the isolated species, *M. gypseum*, *M. cookei*, *T. mentagrophytes* complex, *T. terrestre*, *M. nanum* and *M. fulvum* showed differences regarding the origin of the evaluated soils (valley, mountain or desert soils) and number of isolated dermatophytes. These differences have been attributed by several authors to soil types, vegetation types, and climate-related temperatures [39,14].

Another interesting aspect reported in the literature by Polish researchers is the diversity of soils evaluated in relation to the characteristics of the physical-chemical properties of the soils (soil acidity, presence of humus, calcium carbonate and phosphorus). These authors showed that dominant isolated species were represented by *C. keratinophilum*, which can be isolated within a wide range of pH — 4.5–9.5 [39]. *T. ajelloi*, frequent or very frequent in acid soils [13] was recovered by Polish researchers in neutral or alkaline soils, thus showing intraspecific populational heterogeneity.

Differences can be observed regarding the number of samples evaluated by each group of researchers in different countries, collection techniques employed, and their methodological details, all of which make the comparison of results delicate. Therefore, it is important to report that there are works based on a great number of samples (n = 800), such as the one conducted in Isfahan, Iran, in which 73.5% of keratinophilic fungi were isolated [8], with 16 species belonging to 11 registered genera. As far as dermatophytes are concerned, *M. gypseum* (12.4%) and *T. terrestre* (0.8%) were recovered, as well as other genera representing non-dermatophytes showing different percentages, led by *C. keratinophilum* (31.4%).

Several other studies have demonstrated the presence of this fungus in soils [40,41,5,42]. Some important factors were mentioned by the group of Iranian researchers, namely: depth of soil sampling (3 cm), type of bait used for collection — horse hair, or children's hair, soil pH (greater isolation verified in values of pH fluctuating between 6–9), presence of organic residues and humus, and diferent climates present a direct influence on the percentage and types of isolated dermatophytic and non-dermatophytic fungi [8].

Most of the published studies mention the use of micromorphological techniques in the identification of genera and species of the dermatophytic and non-dermatophytic groups. However, some authors have already used molecular techniques with this same objective, such as Pakshir et al. [9]. This group of researchers evaluated a number of samples similar to this study (n = 196), from 43 parks. In addition to the macro and micromorphological characteristics, DNA sequence analysis was used, amplifying ITS region and sequencing PCR products, producing 411 isolates and 22 identified genera. Most of the isolates were in the pH range of 6–9, where *Fusarium* spp. (23.8%), was the most frequently recovered among non-dermatophytes and *M. gypseum* (1.94%) considering the dermatophytes.

These authors also reported that 66.42%, 32.60% and 0.97% of keratinophilic fungi were isolated from soils presenting pH of 7.01–8; 8.01–9, and 6–7 respectively. In this study, considering the dry and rainy periods evaluated, the pH ranges for both showed values oscillating between 7.8–9.5 for the dry season and 7.7–8.9 for the rainy season, thus highlighting the fact that alkaline soils were present at public day-care centers in Cuiaba, middle-west region of Brazil.

More recently, in 2015, Polish researchers evaluated 104 soil samples from recreational parks in Lodz, and recovered 83 fungal species belonging to 53 genera. Trichophyton genus was more frequently isolated among dermatophytes. In this work, a greater diversity of species was recorded ($p < 0.005$) in autumn than in spring. Forty-five species occurred in both seasons, 19 only in autumn and 26 only in spring [26]. In the study carried out in Cuiabá, a predominance of the isolated dermatophytic fungi was observed in the rainy season (50%) when compared with the dry period (20%).

Several studies have been published focusing on the isolation of fungi in recreational areas such as parks, playgrounds, and squares. Deshmukh and Verekar [43] reported a 63% fungal prevalence in playgrounds and 70% in squares in Poland. Among dermatophytes, *M. gypseum* (9%) was isolated, a species similar to those isolated in sandboxes located in public day-care centers in Cuiaba. In the North-eastern region of Brazil (João Pessoa), Pontes and Oliveira [15] detected the presence of six species of dermatophytes totaling 55.7% of positive samples in school yards, squares and slum areas

(favelas), where *T. mentagrophytes* was the most frequently isolated dermatophyte (37.5%).

In parks in Ahvaz (Saudi Arabia), *T. schoenleinii*, *M. gypseum*, *T. verrucosum* and *T. mentagrophytes* were isolated together with keratinophilic fungi [44].

Non-dermatophytic fungi differ in relation to the isolated species and their percentages in the different geographic locations, it is worth noting that *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Rhizopus* spp. are genera present in several countries, with different climates, soil, or season along which they were recovered [12,33].

As for non-dermatophytic fungi, *Chrysosporium* (*C.*) fastidium was isolated in Cuiabá in the dry period, and *C. tropicum* was the most isolated species (62.2%) in the soils of both schools and parks in India.

Paecilomyces lilacinus was isolated in a larger percentage (27.5%) among non-dermatophyte filamentous fungi in Cuiabá-Brazil, in contrast to the records by Góralska et al., which reported lower and different percentages when evaluating the seasons: autumn 4.02%) and spring (2.70%). Although several genera of non-dermatophytic mycelial fungi (FFND) have been isolated from sand samples contained in recreational boxes, it should be remembered that fungal infections caused by this group usually occur only when faced with individuals with a depressed immune system.

In general, numerous studies referenced in the literature express different percentages, types of genera and similar species regarding the dermatophytic and non-dermatophytic groups, showing variations related with previously mentioned factors (soil types, pH, seasons, dry or rainy periods). The consensus is that despite the different methodologies and number of samples used, the isolation of dermatophyte fungi is present in several countries in recreational parks, public squares, playgrounds, schools and day-care centers. These findings show the need to monitor the quality of soils used for the recreation of children and adolescents, due to the pathogenic potential of the agents that have been isolated from these sites.

Measures to prevent dermatophytoses are usually dependent on the source of infection, that is, if the contact is with zoophilic dermatophytes, the infected animals should be kept away or dewormed. In the case of the circulation of small animals (dogs, cats, rodents, birds) as detected in Cuiabá-MT, the ideal should be the installation of mechanical barriers around the recreational sandboxes. Preventing the access of such animals would be essential in order not to promote the deposition of keratinized hairs and feathers infected with spores of the dermatophytic fungi. Also, when it comes to the clinical and laboratorial diagnosis of dermatophytosis in some children, it should be treated with antifungal agents, and the mother should be instructed for appropriate hygiene measures and also not to share clothes, towels, sheets and personal use objects (combs and hairbrushes) with children living in the same house. It should be noted that these fungal infections are mostly present in populations of low socioeconomic level.

It is fundamental that this information regarding isolated fungal agents (types and percentages) be known by the municipal and state health departments of each geographic region evaluated, aiming at public health measures through sanitary and epidemiological surveillance.

Competing interests

None declared.

Ethical approval

Not required.

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