Original Article

Frequency Distribution of Keratinophilic Dermatophyte Fungi from the Soil of Different Zones in Isfahan Using Morphological and Molecular Methods

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Abstract

Background: Dermatophytes are one of the most important etiologic agents of cutaneous infections in humans and animals. The present study aimed to study the frequency distribution of keratinophilic dermatophyte fungi using conventional and molecular methods in soil of Isfahan city. **Materials and Methods:** In this study, 200 soil samples were randomly selected in three northern, southern, and central parts of Isfahan using hair-baiting technique. The fungi were identified by morphology based on macroscopic and microscopic characteristics of fungi. Furthermore, the sequencing of ITS1-5.8S-ITS2 region of the ribosomal DNA of the 60 randomly isolated fungi was investigated. **Results:** The results of conventional method showed that from a total of 371 fungal colonies, the highest amount of detected colonies was in the central zone (151, 40.26%). Furthermore, in all three areas, the most common detected dermatophyte was *Microsporum gypseum* (38.3%). The results of the molecular analysis showed that *M. gypseum* identified by the morphology method was *Nannizzia fulva*, and also, *Chrysosporium* sp. with the frequency of 30% in morphology method was the second dominated fungus including *Chrysosporium keratinophilum* (42.6%) and *Chrysosporium shanxiense* (21.4%) which confirmed by sequencing method. **Conclusion:** The results showed that keratinophilic dermatophyte fungi including *Nannizzia fulva*, *Chrysosporium sp.,* and *Trichophyton mentagrophytes* are found in the children playgrounds in Isfahan. Therefore, health-care officials should pay more attention to these hygienic issues. *C. shanxiense,* which is found here for the first time in Iran, has been reported only from China.

Keywords: *Dermatophytes, keratinophilic, molecular, morphological, soil*

Introduction

Fungal spores, including keratinophilic dermatophyte fungi, are found in soil all over the world. However, their abundance and population in various zones are different according to the environmental conditions and nutrient materials for the survival and growth of organism.[1] Fungal diseases in humans and animals are important global issues and are largely dependent on social, economic, host-specific conditions, climate conditions, and other factors.[2-5] Contact with infectious fungal spores, for example, dermatophytes can cause skin infections which could be transmitted from soil to humans.[6,7]

Dermatophytes are one of the most common fungal infections in humans and animals. Millions of people worldwide are affected by dermatophytes every year, and as many as 20%–25% of the world population have been infected with dermatophytosis.[8,9]

In nonsexual reproduction classification, dermatophytes are divided into three genera with 41 species: Trichophyton, Microsporum, and Epidermophyton. In the newest dermatophyte taxonomy, Trichophyton includes 16 species, Epidermophyton 1 species, Nannizzia 9 species, Microsporum 3 species, Lophophyton 1 species, and Arthroderma 21 species.[9,10]

The keratinophilic fungi are ecologically, medically, and industrially significant, and therefore, they are increasingly attracting researchers' attention. These fungi are soil inhabitants that produce keratinase and colonize various keratinous substrates and degrade them to their low-molecular-weight components.[11-13]

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At the beginning of the 20th century, separation and cultivation of dermatophyte isolates and species identification were carried out based on morphological characteristics by Raymond Sabouraud, and the taxonomic characteristics of these fungi were determined. He classifies dermatophytes based on the characteristics of sexual and nonsexual production and inclusion of four clinical specimens in the four genera of Achorion, Microsporum, Trichophyton, and Epidermophyton.[9]

In 1934, the Ammonis removed Achorion and only three sexes remained in the class.[9] In 2011, to clarify naming of fungi and avoid different naming in different parts of the world, the naming system of "a fungus a name" was proposed by the researchers which have been used to date with some restrictions. Today, different molecular methods have been used for the taxonomy and identification of dermatophytes, such as random amplification of polymorphic DNA--polymerase chain reaction (PCR), microsatellites, and sequencing.[9] Furthermore, great development has been made with the use of modern molecular methods in the diagnosis of infectious diseases.[14-16]

In terms of epidemiology and ecology, one can understand the ways of spreading the disease, then, transmission chains will be cut, and prevention will be possible to some extent. Identification of keratinophilic dermatophytes to the species level will be used for the better and more effective treatment.

By identifying dominant and native species of keratinophilic dermatophytes, it is easier to examine the underlying fungal diseases associated with these pathogens in Isfahan.[17-19] The current research has been conducted to investigate and identify keratinophilic dermatophyte fungi in areas such as schools, kindergartens, and parks which are playgrounds and resorts for kids and adults in Isfahan.

Materials and Methods

Sampling

This was an applied cross-sectional study of soil of Isfahan, including schools, kindergartens, parks, and playgrounds as a place for children's presence. The statistical population of this study was Isfahan in three parts: central, northern, and southern. A stratified method was used for sampling, and 200 soil samples were randomly collected from volumes to 20 cm long and 20 cm wide and 8 cm deep in plastic bags. After sampling, about 60 g of the soil was poured into a plate, and sterilized distilled water was poured in it to create a uniform and moist surface.[20]

Isolation and morphology identification

We used Vanbreuseghem's hair-baiting technique for isolation of fungi.[21] In this method, the hair of a child under the age of puberty was used as the bait. In this way, some hair is stored in a plate containing soil sample that is wet with sterile distilled water and incubated at 28°C for 1 month.[21] During the incubating time, the soil should not get dry, and the plates were inspected for growth of white colonies on the hairs. Sabouraud dextrose agar medium (Biolife Italiana Sri, Milan, Italy) containing chloramphenicol and cycloheximide (500 mg/L cycloheximide along with 50 mg/L chloramphenicol) was used for the isolation, cultivation, and maintenance of the isolates. Of course, care should be taken not to contaminate the culture media with the saprophytes, especially the *Aspergillus* species. To identify the colony morphology of the fungus, we can lead to the presence of dermatophytes using tease mount or slide culture. To perform the molecular steps, fungal colonies were used to extract their DNAs.[7,20]

Molecular method

To more accurately identify the isolates, from the total of 120 suspected colonies found identical to dermatophytes by morphological methods, DNAs of 60 isolates were randomly sent from all zones for sequencing, but six samples showed not enough qualification and were excluded from sequencing samples.

The sample size flowchart represented in Figure 1 describes the sample size of the isolates and frequency of keratinophilic dermatophytes in both morphology and molecular methods.

The reasons for choosing this number for sequencing include similarity of the isolates in morphology method and save sequencing costs.

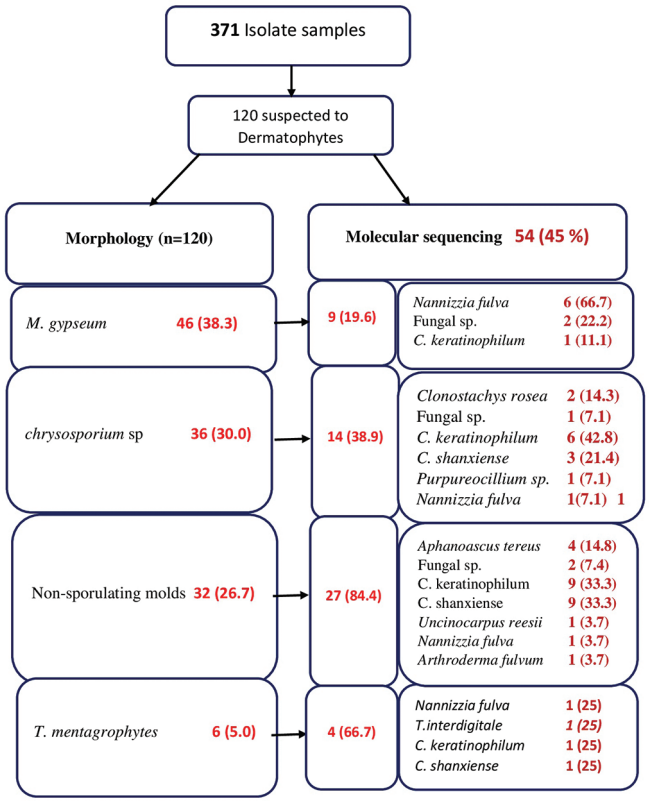

Figure 1: Flowchart of sample size

DNA extraction

DNA extraction was done using fungal DNA extraction kit (DENAzist Fungi DNA isolation kit, Iran) and also phenol–chloroform method in PCR protocols published by Innis *et al*.[22] In this way, a piece of freezed hyphal mass crashed with glass beads in a 1.5-ml tube containing 300 µl of lysis buffer (200 ml sodium chloride 100 mM, 2% Triton X-100, and SDS 1% per liter), 3 µl buffer 10 mM Tris (pH = 8), 1 mM EDTA, and 250 µl phenol–chloroform was transferred to the sterile tube under the hood. Then, with the aid of a micro-dismembrator, the mixture was stirred at a speed of 900 oscillations per minute for 60 s. The DNA was then extracted by a microfuge at a velocity of 5000 rpm from the supernatant, and an agarose gel was used to check and confirm its separation. In later stages, PCR was performed using universal primers, and the amplified DNAs were sent to sequencing for identifying the species for final identification.

Performing polymerase chain reaction

PCR was performed using universal primers (ITS1 and ITS4) with the following sequences: ITS1: 5-TCCG TAGGTGAACCTGCGG 3 and ITS4: 5-TCCT CCGCTTATTGATATGC 3, and A 25 µL volume for a PCR reaction was performed as follows: Premix 12.5 µl, ITS1 primer 0.5 µl, ITS4 primer 0.5 µl, H2O 8.5 µl, and DNA 3 µl.

PCR reaction was carried out using thermocycler with the following condition: denaturation at 95°C for 5 min, 35 cycles of (denaturation at 94°C for 30 s, annealing at 56°C for 45 s and extension at 72°C for 45 s), and final extension 5 min at 72°C. In each set of reactions, negative controls were also considered.

One percent gel electrophoresis was used to view the proliferated band. Dermatophyte proliferated bands were observed at about 600 bp. PCR product purification step for determining proliferated zone sequencing of PCR product was transferred to a 1.5 µm microtube, and 2 times of the absolute alcohol volume was added and placed the microtube in the freezer at −18°C for 0.5 h. Then, it was centrifuged for 10 min with 10000 turns per minutes. Then, the supernatant was removed completely and 30 ml sterilized distilled water was added. To determine the quality of purified DNA, the DNA was run on 1% gel electrophoresis. At the end, 64 random DNA samples from all zones were sent for sequencing. Results of sequencing and determining the species of dermatophyte fungi were identified using the NCBI site which analyses the Blast results of unknown species.

Results

As shown in Table 1, four types of keratinophilic dermatophytes were identified in the soil of schools, kindergartens, parks, and playgrounds of urban areas of Isfahan, among which three genera were identified with the morphological classification.

In this study, a total of 371 fungal colonies (dermatophyte and nondermatophyte molds) from southern, northern, and central zones of Isfahan were isolated [Figure 1]. The highest amount of fungi identified in the central zone included 151 colonies (40.26%), followed by 141 (38%) in the southern and 79 (21.29%) in the northern. The results showed that in all three zones, from the total of 120 suspected dermatophyte isolates, *Microsporum gypsum* (formerly name of *Nannizzia fulva)* was the most

Table 1: Morphological characteristics of identified fungi using conventional methods

Fungal isolate*	Colony color and texture	Colony color reverse	Macroconidia morphology	Microconidia and other characteristics
Microsporum gypseum	Brown, powdery white suede-like to granular, with a deep cream to tawny-buff	Yellow-brown pigment, reddish-brown reverse pigment in some strains	Abundant, ellipsoidal, thin-walled, verrucose macroconidia with 4-6 cells	Numerous clavate-shaped microconidia
Chrysosporium sp.	Powdery white moderately fast growing, flat, white to tan to beige in color, often with a powdery or granular surface texture	Pigment absent or pale brownish-yellow with age	No macroconidia	Hyaline, one-celled conidia are produced directly on vegetative hyphae by nonspecialized conidiogenous cells
Nonsporulation hyphae	Downy, white, pleomorphic	White, creamy to brown	No conidia	Hyaline hyphomycetes with septum
T. mentagrophytes	Central folding, heaped and folded, buff to brown with pleomorphic suede-like to downy, white to cream in color, with a powdery-to-granular surface	Reverse pigmentation is usually a yellow-brown to reddish-brown color	Smooth, thin-walled, clavate-shaped, multicelled, macroconidia	Numerous single-celled microconidia or in dense clusters, spherical chlamydospores, spiral and coiled hyphae

*The genus *Microsporum* is now restricted to just three species: *M. audouinii*, *M. canis*, and *M. ferrugineum*. *T. mentagrophytes* can be distinguished from *T. interdigitale* by its granular appearance on SDA and its microscopic morphology of more spherical microconidia and macroconidia. *M. gypseum*; *Microsporum gypseum*, *T. mentagrophytes*: *Trichophyton mentagrophytes*, *M. audouinii*: *Microsporum audouinii*, *M. canis*: *Microsporum canis*, *M. ferrugineum*: *Microsporum ferrugineum*, *T. interdigitale*: *Trichophyton interdigitale*, SDA: Sabouraud dextrose agar

frequent by conventional methods [Table 2 and Figure 1]. This type of fungus was the highest (56.52%) in the central zone. The frequency of identified fungi in the studied areas showed that the highest (59.01%) amount of fungi was identified in the parks and in the central zone. Schools' soil had the highest 24.59% keratinophilic fungi. In general, in all areas examined, parks had the highest levels of fungi, followed by schools and playgrounds [Table 2]. Table 3 shows the comparison of the result of morphology and sequencing of the isolates with the accession number and amount of homology in Blast software in NCBI (https://blast.ncbi.nlm.nih.gov/).

Discussion

Fungal frequency identified by morphological characteristics is presented in Table 2. As shown in the table, in the present study, the number of morphologically detected keratinophilic dermatophyte fungi was very

Table 2: Frequency distribution of 120 isolates suspected to dermatophytes identified by morphology in different zones of Isfahan

Fungus, *n* (%)	Isfahan zones								
	Central zone, *n* (%)			Northern zone, *n* (%)			Southern zone, *n* (%)		
	Playgrounds	Schools	Parks	Playgrounds	Schools	Parks	Playground	Schools	Parks
M. gypseum 46 (38.3)	5 (50)	6 (40)	15 (41.66)	2 (28.57)	4 (36.36)	7 (30.43)	2 (50)	2 (40)	3 (33.33)
Chrysosporium sp. 36 (30)	3 (30)	4 (26.67)	11 (30.56)	3 (42.86)	4 (36.36)	7 (30.43)	1 (25)	1 (20)	2 (22.22)
T. mentagrophytes 6 (5)	0	1 (6.67)	1 (2.78)	0	1 (9.90)	1 (4.35)	0	1 (20)	1 (11.11)
Nonsporulating fungi 32 (26.7)	2 (20)	4 (26.67)	9 (25)	2 (28.57)	2 (18.18)	8 (34.78)	1 (25)	1 (20)	3 (33.33)
Total 120	10	15	36	7	11	23	4	5	9

M. gypseum; Microsporum gypseum, T. mentagrophytes: Trichophyton mentagrophytes

Table 3: Keratinophilic species identified by morphology and sequencing methods with the accession number and percent of identities, isolated from Isfahan soil

Identification morphology	Accession number	Identification (molecular)	Identity (%)	Identification morphology	Accession number	Identification (molecular)	Identity (%)
M. gypseum	MG573055.1	*N. fulva*	99	*Chrysosporium*	KX462168.1	*C. shanxiense*	99
M. gypseum	KX462168.1	Fungal sp.	99	*M. gypseum*	MG573055.1	*N. fulva*	99
Chrysosporium sp.	KM265103.1	*C. keratinophilum*	99	*T. mentagrophytes*	MG573055.1	*N. fulva*	99
M. gypseum	MG573055.1	*N. fulva*	99	*Chrysosporium*	MG573055.1	*N. fulva*	99
Chrysosporium	KM265103.1	*C. keratinophilum*	99	*M. gypseum*	MG573055.1	*N. fulva*	99
Unknown	KX462168.1	*C. shanxiense*	99	Unknown	KX462170.1	*C. shanxiense*	99
T. mentagrophytes	LT897808.1	*T. interdigitale*	99	*Chrysosporium*	KP269006.1	*C. rosea*	98
Chrysosporium	MG189957.1	Fungal sp.	99	*M. gypseum*	MG189957.1	Fungal sp.	99
M. gypseum	KX668868.1	*N. fulva*	90	Unknown	KM265103.1	*C. keratinophilum*	95
T. mentagrophytes	KX462168.1	*C. shanxiense*	99	*Chrysosporium*	KF367485.1	*Purpureocillium* sp.	99
Unknown	NR_154812.1	*C. shanxiense*	99	*Chrysosporium*	KJ941018.1	*C. rosea*	99
Chrysosporium	NR_154812.1	*C. shanxiense*	98	*Chrysosporium*	KM265103.1	*C. keratinophilum*	99
T. mentagrophytes	KM265103.1	*C. keratinophilum*	98	Unknown	MG573055.1	*N. fulva*	99
Chrysosporium	KM265103.1	*C. keratinophilum*	99	*M. gypseum*	KM265103.1	*C. keratinophilum*	99
Unknown	MG189957.1	Fungal sp.	98	Unknown	AB861820.1	*A. terreus*	98
Unknown	AB861820.1	*A. terreus*	99	*Chrysosporium*	NR_154812.1	*C. shanxiense*	99
Unknown	NR_154812.1	*C. shanxiense*	99	*Chrysosporium*	KM265103.1	*C. keratinophilum*	99
Unknown	AB361653.1	*U. reesii*	90	Unknown	KM265103.1	*C. keratinophilum*	99
Unknown	KM265103.1	*C. keratinophilum*	99	Unknown	NR_154812.1	*C. shanxiense*	91
Unknown	KM265103.1	*C. keratinophilum*	99	Unknown	AB193717.1	*A. fulvum*	95
Unknown	KP147987.1	*A. terreus*	98	Unknown	KM265103.1	*C. keratinophilum*	99
Chrysosporium	KM265103.1	*C. keratinophilum*	99	Unknown	NR_154812.1	*C. shanxiense*	99
Unknown	NR_154812.1	*C. shanxiense*	99	Unknown	NR_154812.1	*C. shanxiense*	98
Unknown	KM265103.1	*C. keratinophilum*	99	Unknown	KM265103.1	*C. keratinophilum*	99
Unknown	NR_154812.1	*C. shanxiense*	98	Unknown	KM265103.1	*C. keratinophilum*	97
Unknown	KM265103.1	*C. keratinophilum*	98	Unknown	KP147987.1	*A. terreus*	95
M. gypseum	MG573055.1	*N. fulva*	93	Unknown	MG189957.1	Fungal sp.	99

M. gypseum; Microsporum gypseum, T. mentagrophytes: Trichophyton mentagrophytes, N. fulva: Nannizzia fulva, C. keratinophilum: Chrysosporium keratinophilum, C. shanxiense: Cypripedium shanxiense, T. interdigitale: Trichophyton interdigitale, A. terreus: Aphanoascus terreus, U. reesii: Uncinocarpus reesii, C. rosea: Clonostachys rosea, A. fulvum: Arthroderma fulvum

limited in comparison to the molecular method in different zones of Isfahan. Among the identified fungi from the soil, three genera were frequently identified by morphological method. However, in a molecular method, by studying the genetic characteristics of microorganisms, we can find a more varied number of fungal species. At the present study we focused on dermatophytes while Anbu et .al in India and Shadzi et al in Isfahan isolated keratinophilic fungi , so the results are not the same.[6,23]

It is worth noting that the sequencing results of *Chrysosporium* showed that of 54 DNA sample isolates, more than half of them identified as the genus of *Chrysosporium*, including 17 strains (56.6%) of *Chrysosporium keratinophilum* and 13 strains (43.4%) of *Chrysosporium shanxiense* [Figure 1 and Table 3]. Therefore, nonsporulating hypha in conventional method belonged to this genus.

The present study revealed that the newly recognized species of *Chrysosporium shanxiense* was reported for the first time in Iran. The name of the s*hanxiense* originates from Shanxi a place first this fungus identified by Zhang *et al*. in 2016 in China.[24] The colonies of *Chrysosporium shanxiense* are formed at 26°C for 14 days. The colonies have a rough, coarse, and white powdery surface and sometimes surrounded by several aerial mycelium.[24] In this study, *Chrysosporium shanxiense* was identified with a frequency of 43.4% using molecular method.

In a study done by Shadzi *et al*., in 2002, on isolated keratinophilic fungi from the dust of schools and parks in Isfahan, 214 isolates of keratinophilic fungi in seven genera were identified morphologically, and the most common species was *Chrysosporium keratinophylum* with frequency of 54.2%, which means that more than half of the keratinophilic isolates from the parks and schools were identified *Chrysosporium*. The most common dermatophyte frequently isolated in classrooms and parks was *Microsporum gypseum,* followed by *M. canis.* Their study focused on morphology we found the same about *M. gypseum* but we did not detect any *M. canis*.[23]

Here, the differentiation of *Chrysosporium* species performed more accurately by DNA sequencing method. In conventional methods, *Chrysosporium* species have a high morphological similarity of fruiting bodies, so many misidentified isolates could be mistakenly reported or collected in literature or in fungal collections. Actually, these misidentifications are due to the method of identification and new classification or nomenclature of fungi. These taxonomical changes are due to the development of new molecular and reclassification of the microorganisms overtime.

Some of *Chrysosoporium* species such as *C. keratinophilum*, *C. tropicum,* and *Chrysosporium* sp. have been reported as agents of cutaneous and systemic mycoses.[25] Abundance of these geophilic isolates might provide the risk of transmission of infection to humans and animals.[17]

In the current study, more than half (55.5%) of detected fungi were identified to be *Chrysosporium* by molecular method. *M. gypseum* (formerly name of *N. fulva*) was the most frequent keratinophilic dermatophyte species detected by morphology method in three regions of Isfahan. Eight of detected *N. fulva* strains showed 99% identity to *N. fulva* (accession no: MG573055.1) but one strain to KX668868.1.

We also isolated four more genera of keratinophilic fungi from the soil of Isfahan. *Aphanoascus terreus, Clonostachys rosea, Purpureocillium sp.,* and *Trichophyton interdigitale* were the genera which identified as shown in Table 3 and Figure 1. *Aphanoascus* is an ascomycetous mold with keratinophilic activity. This genus with 12 species is not a dermatophyte fungus and does not responding to antifungal drugs. *Aphanoascus* is very similar to *Chrysosporium,* and some of the isolates have been detected from onychomycosis infections[26,27] However, at the present study, we found four species of *Aphanoascus terreus* by molecular method.

Moallaei *et al*. in 2006 investigated the keratinophilic fungi from various areas of forests and farmyards in north of Iran. They isolated 375 colonies from 50 soil samples. In their investigation, McNemar's test showed that non-keratinolytic fungi were dominant among the isolates ($P < 0.05$). *Anixiopsis stercoraria* was dominant keratinophilic fungus (21.84%), followed by *Arthroderma cuniculi* (12.04%), *C. keratinophilum* (8.4%), *Trichophyton vanbreuseghemii* (7.84%), and *Microsporum gypseum* (1.2%).[11] The growth rate and sporulation of fungi in soil is affected by environmental conditions like humidity, soil pH, temperature and nutritional factors. In north of Iran with the high humidity and rainy weather has more variant soil microorganisms and fungi.[28,29] Isfahan is located in central of Iran which remains hot during the summer with low humidity and moderate temperatures at night and during the winter, days are mild while nights can be very cold.

Pakshir *et al*. in 2013 studied and isolated the keratinophilic fungi from the soil of Shiraz Parks. In this study, 196 soil samples were collected from 43 parks in Shiraz using the hair-baiting method to detect all keratinophilic fungi. The identification of fungi was conducted based on morphological and molecular methods, and they found 22 genera from 411 colonies.[20] The soil of parks have been enriched by man and possess more fungal elements. At the present study, we detected more keratinophilic dermatophytes from the parks of Isfahan

In Kumar *et al*. research, 48 soil samples were collected from 12 garbage waste soil. They found a total of 64

colonies including 7 genera of different keratinophilic fungi. *Penicillium chrysogenum* (15.62%) was the most dominant and *Chrysosporium sp.* with a frequency of 4.69% was the least fungi. Obviously, the contrary result about the frequency of *Chrysosporium* sp. is the source of waste soil and also the method of investigation.[30]

Deshmukh and Verekar examined the prevalence of keratinphilic and related dermatophyte fungi in various areas of Kerala state in India. From a total of 158 soil samples, they found eight genera of keratinophilic fungi. The genus Chrysosporium including *C. indicum* (20.25%), *C. keratinophilum* (6.96%), C. *lobatum* (1.26%), *C. pannicola* (1.26%), and *C. tropicum* (5.06%) was the predominant species. The dominant dermatophyte was reported by *M. gypseum* complex (12.65%). Similar to our research, they found that *M. gypseum* showed the highest distribution, and in contrary our results, they found in four another different species of Chrysosporium in addition of *C. keratinophylum*.[31]

Based on a recent multilocus phylogenetic study, the taxonomy of the dermatophytes has been changed. The genus Microsporum is now restricted to three species: *M. audouinii, M. canis,* and *M. ferrugineum*. Two geophilic species including *M. gypseum* and *M. flavum* transferred to the genus of Nannizzia.[9,10]

In a study done by khosravi *et al*. on domestic animals in Iran, *M. gypseum* and *Trichophyton mentagrophytes* were the most frequent species isolated from rabbits and squirrels, respectively. In addition, they isolated *M. gypseum* from cat, dog, goat, horse, and rabbit. Although *M. gypsum* is not frequently isolated from human, it currently isolated from domestic animals. At the present study, *M. gepseum* was the most frequent dermatophytes, so in the people which adopt animals, they may be at risk for dermatophytosis with this fungus.[32]

Soil are considered as the most complex media of microbial residents, including fungi. Some fungi of the soil are associated with human and animal diseases and cause chronic problems. The soil differ from the chemical composition and support the growth of a particular fungal flora; however, most of its fungi grow natively in the soil. Fungi have a high adaptive ability in terms of lifestyle and conditions as there are a variety of metabolic pathogens in them. The diversity and composition of the microbiome, survival, compatibility, and fungal resistance in the soil change profoundly across environmental conditions. Fungal diversity has been greatly facilitated by the development of different methods, which has enabled the identification of isolates with sequencing thousands of DNA samples.[28] The presence of fungal species identified in the present study may be due to soil conditions and general environmental factors governing soil in the zones of Isfahan.

Conclusion

The comparison of morphological and molecular methods for the identification of fungi showed that the molecular method seems more accurate in nonsporulating molds. The results can be used by decision-makers to prevent and manage the factors responsible for the spread of pathogenic fungal spores. In addition, it can be argued that the amount of keratinophilic dermatophyte fungi including *Nannizzia fulva*, *Chrysosporium,* and *T. mentagrophytes* is found in the soil of children's playgrounds in the city of Isfahan. Therefore, health-care officials should pay more attention to these hygienic issues.

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Conflicts of interest

There are no conflicts of interest.

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