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RESEARCH ARTICLE

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Prevalence of superficial-cutaneous fungal infections in Shiraz, Iran: A five-year retrospective study (2015–2019)

Hossein Khodadadi[1] | **Kamiar Zomorodian**[1,2] | **Hasti Nouraei**[1] | **Zahra Zareshahrabadi**[1] | **Sajjad Barzegar**[3] | **Mohammad Reza Zare**[3] | **Keyvan Pakshir**[1,2]

[1]Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[2]Basic Sciences in Infectious Diseases Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[3]Department of Chemical Engineering, School of Chemical and Petroleum Engineering, Shiraz University, Shiraz, Iran

Correspondence
Keyvan Pakshir, Department of Parasitology and Mycology, Basic Sciences in Infectious Diseases Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
Emails: pakshirk@gmail.com; pakshirk@sums.ac.ir

Abstract

Background: Superficial and cutaneous fungal infections are common in tropical areas. The aim of this study was to provide a basic database of superficial and cutaneous mycoses and the most common etiological agents among patients.

Methods: Between 2015 and 2019, a total of 1807 patients suspected of superficial and cutaneous mycosis referring to the mycology laboratory of Shiraz medical school, Fars, Iran were evaluated. Specimens were taken from the patients' affected area, and clinical samples were examined by direct microscopy and culture. The epidemiological profile of the patients was collected.

Results: A total of 750 patients were confirmed with mycoses. Positive samples totaled 750 cases consisting of the nail (373/49.7%), skin (323/43%), head (47/6.26%), and mucosal membrane (4/0.5%). The yeasts group included 304 *Candida* spp. (70.3%), 123 *Malassezia* spp. (28.47%), and 5 *Rhodotorula* spp. (1.1%). The filamentous fungi were distributed as 34.8% dermatophytes and 7.5% non-dermatophyte. The clinical types of dermatophytosis were tinea unguium (110/261), tinea capitis (50/261), tinea pedis (48/261), tinea corporis (37/261), and tinea cruris (16/261). Non-dermatophyte molds included *A. flavus* 17, *A. niger* 4, *Aspergillus* spp. 15, *Penicillium*. 10, *Fusarium* 6, *Mucor* 2, *Stemphylium* 1, and *Alternaria* 1.

Conclusion: This study provides useful data for the study trends of superficial and cutaneous fungal infections in a specific area. The mycological data confirmed higher incidence of candidiasis (mainly onychomycosis) and dermatophytosis in patients affected by fungal pathogens, which helped to better understand the epidemiological aspects of these mycoses.

KEYWORDS
dermatophyte, fungal infections, non-dermatophyte molds, yeast

1 | INTRODUCTION

Fungal diseases are common and cause widespread diseases, especially in tropical countries. Superficial fungal infections (SFI) are the most frequent ones and are usually limited to the skin layers, hair, or nails. Superficial and cutaneous fungal infections are the most common dermatological diseases worldwide, with a high prevalence mostly caused by dermatophytes, yeast, and to a lesser extent, non-dermatophyte filamentous fungi (NDFF).[1] The prevalence rate of SFI worldwide has been found to be 20%–25%.[2] Dermatophyte fungi invade the keratinized tissue such as the skin (epiderm) and its appendages. In this group, pathogenic species are anthropophilic, zoophilic, and geophilic fungi belonging to three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*; their new classification is as follow: *Trichophyton*, *Epidermophyton*, *Nannizzia*, *Paraphyton*, *Lophophyton*, *Microsporum*, and *Arthroderma*.[3] Dermatophyte species are transmitted through exposure by direct contact with the soil, animal, and infected person.[4,5] The most clinical manifestations include tinea capitis, tinea corporis, tinea pedis, tinea unguium, and tinea faciei.[6] Besides dermatophytes, SFI are often caused by yeasts groups mostly belonging to the genus *Candida*, especially *C. albicans*. It is crucial that the prevalence of non-*Candida albicans* (NCA) species as causative agents has increased in recent years.[7] Also, sometimes dematiaceous yeasts could cause superficial mycosis.[8] The clinical manifestations of superficial and cutaneous *Candida* infection vary, such as oral thrush, oropharyngeal candidiasis, vaginal candidiasis, cutaneous candidiasis, paronychia, and onychomycosis, and chronic mucocutaneous candidiasis.[9,10]

Malassezia spp. are residents of skin microflora, which cause diseases such as pityriasis versicolor (PV), *Malassezia* folliculitis, and seborrheic dermatitis.[11] In addition, in the recent literature, there has been an increase in published data on the pathogenic potential of non-dermatophyte molds in SFI, mainly caused onychomycosis.[12] Notably, some superficial bacterial infections of the skin and hair are frequently mimicking fungal infections, including erythrasma and trichomycosis axillaris.[13,14] Erythrasma is a superficial bacterial infection of the skin caused by *Corynebacterium minutissimum*. It is characterized by well-demarcated scaly patches with reddish-brown color in the body's intertriginous sites and causes itching, scaling, and erythema. It is often asymptomatic. However, it can be differentiated from dermatophytosis and candidiasis by the distinctive 'coral-red' fluorescence under Wood's lamp illumination.[14]

The diagnosis of SFI is usually based on using conventional methods as microscopic and macroscopic examination. Nowadays, the prevalence of fungal causative agents that causes SFI is changing and mostly depends on the geographical, ecological, and socio-economic conditions. Although SFI are not life-threatening diseases, they can significantly affect the quality of a patient's life.[7] According to previous epidemiological studies, an increasing number of predisposing risk factors are associated with fungal infections of the skin, hair, and nails, such as low-level socio-economic conditions, poor personal hygiene, artificial nails, metabolic disorders, and biological therapy.[15] This study aimed to determine the most common superficial and cutaneous fungal infections and related causative agents in patients referring to Shiraz medical mycology diagnostic laboratory in Shiraz city, Iran.

2 | MATERIALS AND METHODS

2.1 | Clinical specimens and sample processing

During five years from January of 2015 to December of 2019, a total of 1807 samples from patients suspected of SFI who were referred to the mycology laboratory of Shiraz Medical School were taken. For each patient, general information such as age, sex, site of infection, underlying disease, and previous history of drug usage was recorded.

The infected areas and lesions were cleaned before sampling. Using a sterile hair plugger, nail clipper, and scalpel, the infected hair, nail, and scales were collected. All the samples were collected in a sterile petri dish. A portion of samples was used for direct examination, and the other portion was used for cultivation in mycological media or staining, depending on the kind of fungal infection.

The only direct mycological examination was used for suspected samples of pityriasis versicolor (PV) and erythrasma.[16] In cases that were suspected to have a superficial or cutaneous bacterial infection, specimens were collected by scraping of the affected areas. The direct examination was performed for all specimens and identified based on the suspected samples' microscopic features, which were stained with methylene blue or Gram stain. Wood's lamp was used to help identify suspicious fungal/bacterial lesions.[14]

2.2 | Direct examination

All the samples were collected on a sterile slide, and we used potassium hydroxide (KOH) 10%–20% or lactophenol (for hair samples) solutions for direct examination. The details regarding the hyphae or branching hyphae, spores, budding cells, and pseudohyphae were noted. Suspected lesions of PV were collected by using scotch tape over the affected area, which should show numerous hyphae with round yeasts in grapelike clusters, which presents a spaghetti-and-meatballs pattern.[16] The collected samples were stained by methylene blue or Gram stain in erythrasma and PV suspected cases.[13] In addition, with wood's lamp, the bright coral red, and yellowish fluorescence indicated erythrasma and PV, respectively.

2.3 | Culture

The samples obtained were cultured in two series: sabouraud's dextrose agar (Merck) containing chloramphenicol (50 mg/L) + cycloheximide (500 mg/L) (SCC) and sabouraud glucose agar without cycloheximide (SC). The incubation temperature was 25–35°C for four weeks, and the cultures were monitored daily for observation of any colony-forming growth. The mucoid colony represented the yeast fungi, and the mold colony revealed the filamentous fungi.

2.4 | Identification of isolates and fungal infection

The identification of the fungal genus was performed based on macroscopic texture and appearance of the colonies and microscopic features of fungi morphological characteristics under light microscopy (40 ×). For macroscopic identification, the texture and topography, margins and colors of colonies, obverse and reverse colony pigments, and growth time were evaluated. Also, tease mount and slide culture methods were applied for microscopic identification. The shape of micro- and macro-conidia and accessory structures were used for better detection.[17] The presence of yeast, pseudohyphae, and budding forms showed a yeast infection. The presence of septate, nonseptate hyaline or dematiaceous hyphae, and arthroconidia was classified as a mold infection.[16]

2.5 | Statistical analysis of data

A descriptive analysis of the patients' demographical data and clinical characterization was conducted, and the data were entered into the Microsoft Excel and SPSS software for data analysis. A Chi-square test and Fisher's exact test were used to determine the relationship between the identified genus and their demographical data. The p-value ≤ 0.05 was considered significant.

2.6 | Ethical approval

This project was found to be in accordance with the ethical principles and the national norms and standards for conducting Medical Research in Iran and approved by the research ethics committee. (IR.SUMS.REC.1399).

3 | RESULT

In the present study, throughout 5 years (2015–2019), 1807 samples including 941 skin, 696 nails, 161 hair, and nine mucosal specimens were collected from the lesions of patients suspected of superficial mycoses. Patients included 811 (44.9%) men and 996 (55.1%) women, with a mean age of 33.25 (1–92) years. The highest and lowest age groups were 21–40 years with 786 (43.5%) and over 60 years with 196 (10. 8%) patients, respectively. Superficial and cutaneous fungal infections were diagnosed in 750 (41.5%) patients by clinical and mycological examination. Among the confirmed patients, the highest age group was 21–40 years, and then the elderly over 60 years old (12.4%) comprised the at least age group (Table 1). In this study, we observed the low prevalence rate of SFI in children. More details about age and gender in SFI are presented in Table 1. It is noteworthy that 18.2% of all positive KOH specimens had negative cultures. In the present study, 57.7% (n = 432) of the isolates belonged to the yeast group, including 304 *Candida* spp. (70.3%), 123 *Malassezia* spp. (28.47%), and 5 *Rhodotorula* spp. (1.1%). Among the patients with candidiasis, women (66.1%) were significantly more than men (33.9%) (p < 0.001). The most common clinical forms of candidiasis were onychomycosis (77.5%), followed by cutaneous (20.5%) and mucocutaneous candidiasis (2%). The second most common SFI was dermatophytosis (n = 261, 34.8%), and tinea unguium with 42.1% (110/261) was the most prevalent type of dermatophytosis, followed by tinea capitis (19.1%, 50/261), tinea pedis (18.3%, 48/261), tinea corporis (14.1%, 37/261), and tinea cruris (6.1%, 16/261). In this study, etiological agents of dermatophytes were 20 (7.6%) *T. mentagrophytes*, 11 (4.2%) *T. rubrum*, 5 (1.9%) *Epidermophyton floccosum*, 15 (6.6%) *Microsporum canis*, 3 (1.27%) *T. verrucosum*, 2 (0.84%) *T. violaseum*, and 2 (0.84%) *M. gypseum*. We also identified 53 (20.3%) *Trichophyton* spp., 22 (5.1%) *Microsporum* spp., and 129 (49.4%) unknown dermatophytes. Remarkably, *Trichophyton* species (67.4%) was the predominant genus among dermatophytes isolates. Non-dermatophytic filamentous fungi were identified in 56 (7.5%) onychomycosis samples that included *A. flavus* complex 17 (30.3%), *Aspergillus* spp. 15 (26.7%), *Penicillium* spp. 10 (17.8%), *Fusarium* spp. 6 (10.7%), *A. niger* complex 4 (7.1%), *Mucor* spp. 2 (3.57%), *Stemphylium* sp. 1 (1.78%), and *Alternaria* sp. 1 (1.78%) (Table 2).

In the present study, 53 adult patients (34 men and 19 women) fulfilled the erythrasma diagnostic criteria. The presence of erythrasma infection in male was significantly more than female patients (p-value: 0.005). In addition, trichomycosis axillaris was detected in the axillary site of two female patients after gram stain smears.

4 | DISCUSSION

As the most widespread group of mycotic diseases, the distribution of SFIs and their causative agents are different according to the reports from other countries. The major clinical categories of SFIs are dermatophytosis, superficial candidiasis, and pityriasis versicolor (PV).[18] All these conditions are treatable and preventable. The epidemiology of

TABLE 1 Distribution of superficial and cutaneous fungal infection according to age and gender

Age in years	Male	Female	Total	Most infected site
0–20	91 (57.0%)	67 (43.0%)	158 (21.0%)	Head (28.8%)
21–40	137 (41.1%)	206 (58.9%)	343 (42.9%)	Body (33.8%)
41–60	63 (38.2%)	107 (61.8%)	170 (23.8%)	Foot nail (27.3%)
>60	37 (46.9%)	42 (53.1%)	79 (12.4%)	Finger nail (32.7%)
Total	328 (43.7%)	422 (56.3%)	750 (100%)	

TABLE 2 Frequency of fungal isolated in superficial fungal samples

Fungi isolated	*N*	%
Candida spp.	304	40.5
Malassezia spp	123	16.8
Rhodotorula spp.	5	0.7
Trichophyton spp.	89	11.8
Microsporum spp.	29	3.9
Epidermophyton.	15	2
Unknown dermatophyte	129	17.0
Aspergillus spp.	36	4.8
Fusarium spp.	6	0.8
Penicillium spp.	10	1.3
Mucor spp.	2	0.2
Black fungi	2	0.2

Note: N, number of isolates; %, percentage of the different species

SFIs can be changed by many factors such as geographic, climate, historical factors, immigration, wars, healthcare quality, interventional medicine, and level of society social factors of the region.[5]

Pathogenic fungi are usually identified by conventional and phenotypic methods in most clinical laboratories and hospitals. Identification and confirmation of fungal isolates by routine clinical diagnostic analysis are still commonly used although these methods have limitations for identifying the species level.[16,19]

As mentioned before in this study, 18.2% of all specimens that had positive KOH had negative cultures that were probably due to previous prescription of antifungal agents (6.7%), antibiotics (0.5%), steroids (4.7%), or other chemical materials. Theoretically, prior treatment with antifungals can affect the culture results.

In previous studies, SFIs have been categorized as the most frequent infections, affecting more than 20%–25% of the world population[20]; also, in Iran, this range was from 9.16 to 52%.[21–23] Here, we observed the incidence of these diseases to be 41.5%. These differences among the studies could be due to the ecological conditions, sample size, and methodology of identification.[5,14]

Some researchers indicated that the incidence of SFI was high among the patients aged under 20 years,[18] but we observed that it was most prevalent among older patients (21–40 years old); the same results were found in other studies in India and South Korea.[24,25] It may be because of the fact that in these years people have more job activities, so they have more chances of exposure to fungal agents, and also they care about their health and pay more attention to appearance and beauty.

Koksal et al.[26] in Turkey and Elgart et al. in China have reported that men and women are equally affected by SFI,[18] while in our study, a higher incidence was confirmed in women, with a sex ratio of 1.2. In line with the current study, Egyptian, South Korean, and Brazilian women were more affected by SFI.[16,25,27] On the contrary, Gamage et al.[28] and Berenji et al.[29] reported that the prevalence of SCFI was higher in men than women. Several factors can affect gender prevalence, such as personal hygiene, occupational activity, and exposure to the contamination agents.[5,30]

Regarding epidemiological characteristics, we identified some risk factors for SFI such as diabetes mellitus, long-term treatment with antibiotics and immunosuppressives, and steroid drugs, which were significantly more common, especially in the candidiasis patient groups.

In the current study, the yeast genus was the most frequent type of isolates and accounted for 57.7% (n = 432) of all positive samples, followed by dermatophytes (34.8%) and NDFF (7.5%). According to our findings, candidiasis, with a prevalence of 40.5%, was reported as the most frequent type of SFI. In comparison to other studies, a higher rate of candidiasis in Brazil (82.9%) and in Southeastern Serbia (57%) was confirmed.[7,16]

We reported that dermatophytosis was the second common type of SFI (34.8%), which is in line with previous studies.[7] However, in epidemiologic studies in China, Turkey, and Germany, 82%, 74%, and 83.2% of dermatophytosis were reported, respectively.[26,28,31] In this study, the nails were the most affected anatomic site in the patients, as similarly reported in Egypt and Southern China.[18,27] Some studies showed that tinea capitis,[32,33] tinea corporis,[22,34–36] tinea pedis,[37] and tinea cruris [23,30] were the predominant clinical forms of dermatophytosis. Although tinea unguium is not a life-threatening condition, it is associated with difficulties in performing hand activities and is a cosmetic problem that affects the quality of life. In addition, there are some reports about poor response to cure tinea unguium or even the emergence of resistant strains to antifungal agents; for example, terbinafine-resistant *T. rubrum* isolates have been reported. Therefore, diagnosis and efficient treatment of tinea unguium are essential.[38]

The epidemiological pattern of dermatophytoses in our study showed that *Trichophyton* species were the most prevalent agents among the isolates, and this finding was similar to other studies.[16,18] *Trichophyton mentagrophytes*, *T. rubrum*, and *M. canis* were the three most commonly dermatophytes isolated species in the current study. We observed that *T. mentagrophytes* was the predominant dermatophyte in this study, which is in line with the Zareshahrabadi et al.[5] report but in contrast with other findings which showed that *T. interdigitale* was the most prevalent species.[35] In this study, tinea cruris (6.1%) cases were the least reported among the dermatophytosis clinical manifestation, and *E. floccosum* was obtained from all cases. In our study, one of the possible reasons for determining the low prevalence of tinea cruris was using an empirical approach for healing without a laboratory confirmation examination, and self-therapy even without a prescription. In Iran, for treating dermatophytosis, terbinafine, fluconazole, itraconazole, and griseofulvin are the available antifungal drugs that are routinely used.[35] According to the recent increase in dermatophytosis and emergence of antifungal drug resistance, performing reliable antifungal susceptibility testing is essential, particularly for chronic and recurrent dermatophytosis.[39,40]

Species identification was often difficult by classical techniques, and sometimes, the results were released only at the genus level as; for instance, we report only *Trichophyton* spp.

Moreover, a dermatophyte culture can be time-consuming as it requires more than 12–20 days for better growth and observation of typical characteristics. Molecular techniques for identifying the fungi species are faster and more sensitive. However, they are not yet an alternative used in the routine clinical diagnosis of superficial mycoses due to the cost-effectiveness and the need for more professional expertise. Commercial molecular tests are designed for rapid diagnosis of SFI, but they are expensive for most general laboratories.[12]

Onychomycosis was observed with a high frequency in this study, and, notably, this disorder included 18%–50% of all nail infections in the world.[41,42] The *Malassezia* spp are human skin microbiome and the causative agent of PV. It was common in our study area due to the climate condition and some limitations in diagnostic species for this genus. The culture was not required. Also, identifying *Malassezia* yeast at the species level does not have much value to define the treatment.[11] In our study among 123 *Malassezia* spp., there was a significant difference between male and female patients in affecting PV (p-value: 0.01). These findings may be related to higher sebaceous glands secretion in males.

Although, in the current study, NDFF was isolated from onychomycosis, dermatophytes were the predominant genus among filamentous fungi. Non-dermatophytes mold is commonly found as soil and phytopathogens saprophytes. Among the confirmed NDFF causing mycoses in our study, we isolated *Aspergillus* spp. more than another genus with worldwide distribution.[27]

In the present study, 53 cases of erythrasma from the groin were detected. These findings were similar to previous studies[14,43]; it was more prevalent in men, which was statistically significant. Multiple factors enhance predisposition to erythrasma, such as geographical region, hyperhydration, poor hygiene, diabetes, advanced age, and obesity. Erythrasma is a common condition and can be misdiagnosed with tinea cruris, PV, and candidiasis. This disorder persists if not diagnosed and appropriately treated.[14] Identification of the etiological agent is essential to help physicians with appropriate therapies, and it is crucial to establish the fungal epidemiological trend in a specific area. In addition, this study can determine fungal epidemiological patterns that can facilitate the choice of preventive procedures. Notably, an inaccurate SFI diagnosis can lead to inappropriate treatment. In this study, one of our limitations was lack of possibility to use molecular methods for better identification of the agents, so although they are cost-effective, these methods could be helpful in reaching accurate results.

5 | CONCLUSIONS

Superficial-cutaneous fungal infections are an important dermatological disease and are among common diseases worldwide. Our study with a high number of biological samples analyzed in 5-year long retrospective research helped establish the epidemiological pattern of the mycoses. It demonstrated the most prevalent mycoses and fungal agents in patients who were referred to mycological laboratories of Shiraz Medical School. The present study results can help increase knowledge about the fungal agents, SFI patterns, epidemiology of these infections, and their appropriate treatment.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study were supplied by Shiraz University of Medical Sciences under license and so cannot be made freely available. Requests for access to these data should be made to Keyvan Pakshir, pakshirk@gmail.com.

ORCID

Keyvan Pakshir https://orcid.org/0000-0001-7809-4690

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