

Epidemiological Aspects of Dermatophytosis in Khuzestan, southwestern Iran, an Update

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Abstract Dermatophytosis is among the most common superficial mycoses in Iran. The purpose of this report was to update the clinical and mycological features of human dermatophytosis in the Khuzestan, southwestern Iran. In the framework of a one-year survey, a total of 4120 skin, hair and nail samples obtained from the outpatients with symptoms suggestive of tinea were analyzed by using direct microscopy, culture and molecular identification methods. Strains isolated from cultures were subjected to amplification of the nuclear *rDNA ITS* regions in a PCR assay followed by an early established RFLP analysis. For confirmation of species identification, 100 isolates as representatives of all presumable species were subjected to ITS sequencing. Infection was confirmed in 1123 individuals (27.25 %) in the age range of 1–89 years by direct microscopy and/or

culture including 603 males versus 520 females. Frequencies of infections were the highest and the lowest in age groups of 21–30 and 11–20 years, respectively. Tinea corporis was the most prevalent clinical manifestation followed by tinea cruris, tinea capitis, tinea manuum, tinea pedis, tinea unguium, tinea faciei and tinea barbae. *Trichophyton interdigitale* (58.7 %) was the most dominant isolate followed by *Epidermophyton floccosum* (35.4 %), *Microsporum canis* (3 %), *T. rubrum* (1.5 %), *T. species of Arthroderma benhamiae* (0.5 %), *T. tonsurans* (0.3 %) and *T. violaceum* (0.3 %). Other species included *M. gypseum*, *M. fulvum* and *T. verrucosum* (each one 0.1 %). Such a high occurrence of infection with *T. interdigitale*, which has not been reported from Iran, is due to the use of accurate molecular methods based on new species concept in dermatophytes. The

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prevalence of dermatophytoses caused by zoophilic species remarkably increased and *Trichophyton* species of *A. benhamiae* has emerged as a new agent of dermatophytosis in southwestern Iran, while infections due to anthropophilic species, except *E. floccosum*, took a decreasing trend.

Keywords Tinea · Dermatophyte · Epidemiology · ITS · Khuzestan

Introduction

Dermatophytes are a group of keratinophilic molds with worldwide distribution and encompass the genera *Trichophyton*, *Microsporum* and *Epidermophyton*. They can invade and infect the nails, hairs and stratum corneum of the skin and cause spectrum of common conditions, medically termed as dermatophytosis (ringworm or tinea), that inflict health problems to both human and animal [1, 2]. Dermatophytic infections are known to be not life-threatening as much as of invasive mycoses and usually affect normal individuals; however, for appropriate treatment and control of potential sources of infection the accurate distinction of causative agents is essential [2, 3]. The epidemiological profiles of dermatophytoses are consistently evolving due to alterations in lifestyle, socioeconomic and climate condition, expansion of tourism and migration activities [3, 4]. Other important issues are change in the taxonomy of dermatophytes, discovery of some novel and long-forgotten species that ensued following the introduction of a new dermatophyte species concept [3, 5–7]. According to reasons cited above, to perceive the coming epidemiological trends of dermatophytoses, regular and successive surveillance of these infections and relevant agents is necessary. In this regard, recent advancements in molecular biology have introduced impressive, fast and reliable methods reliant to DNA sequence for identification and screening of dermatophytes [3, 8–10]. A recent DNA-based query implies that some alterations are occurring in the frequency rate of fungal species causing clinical variants of dermatophytosis in Iran [3]. The epidemiological aspects of dermatophytosis were previously characterized in Khuzestan, southwestern Iran [11]. In this survey, we sought to determine the current trend of

this infection and related causal agents in this province by using a set of sequence-based procedures.

Materials and Methods

Patients and Isolates

Over a one-year prospective survey, from May 2013 to May 2014, the outpatients from Ahvaz (the center) and other cities of Khuzestan Province, suspected of dermatophytosis referred to our medical mycology laboratory (Iran-Zamin diagnostic laboratory) were included in the study. All individuals were requested to fill in a personal information questionnaire to inquire data related to infections, e.g., age, gender, history of animal's contact and so forth. The ethic permission (IR.AJUMS.REC.1394.443) was granted by the Ethic Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The clinical samples including skin ($n = 3036$), hair ($n = 734$) and nail ($n = 350$) were aseptically taken from each patient by scrapping. One portion of each sample was mounted in 15 % KOH solution and then examined microscopically to verify the presence of fungal elements. The remaining part of each specimen was inoculated into Mycosel Agar (BD Diagnostics, NJ, USA), incubated at 28 °C and checked daily up to a total of 4 weeks for the dermatophyte growth. All of the grown isolates were preliminary screened based on the gross- and micromorphology of the colony, and strains identified as dermatophytes were subjected to further molecular analysis.

Identification of the Isolates

In general, we applied a two-step enzymatic digestion of the ITS rDNA products for identification of the isolates, as previously described [3]. For nucleic acid isolation, a small piece of each fresh colony was mechanically milled in the presence of 0.5-mm glass beads and lysis buffer in a homogenizer (SpeedMill Plus) and the DNA was purified by using of the manufacturer's protocol (Analytik Jena, Jena, Germany). The 5.8S subunit of rDNA and its flanking internal transcribed spacers 1 and 2 were amplified using the primer pair ITS1 and ITS4 [12]. Thereafter, the obtained amplicons were subjected to digestion with the *MvaI* and if needed *BsrI* (Thermo Fisher

Scientific, Waltham, MA, USA) or *MaeIII* (Roche Diagnostics, Mannheim, Germany) restriction enzymes, according to the manufacturers' instructions. For identification of isolates, the restriction products were electrophoresed through 2 % gel agarose, and size of digested fragments was compared with that illustrated in the previous report [3].

Sequencing

To distinguish those isolates where morphology and RFLP were not helpful, and moreover to introduce the sequence data of Iranian dermatophytes strains, a total of 100 isolates were subjected to sequencing. Briefly, the ITS products were cleaned using a QIAquick purification kit (Qiagen GmbH, Hilden, Germany) and then sequenced on an ABI PrismTM 3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA) with the ITS1/ITS4 primers. The obtained sequences were edited and blasted against CBS database (www.cbs.knaw.nl/dermatophytes) using standard criteria for a significant match.

Statistical Analysis

The effect of sex and age variables on the incidence of different infections was tested by Chi-square (χ^2) test in IBM SPSS Statistics (version 20). Two-sided p value was calculated, and the level of statistical significance was set at $P < 0.05$.

Results

Totally, in 1123 individuals (27.25 %) including 603 males versus 520 females the infection was confirmed by direct examination and/or culture. The positive samples consisted of 910 skins, 135 hairs and 78 nails. Majority of the patients were from suburban or rural areas and engaged in agricultural activities with history of dealing with poultry or livestock such as cow, bufflehead, sheep, goat and horse.

In Tables 1 and 2, the frequency of dermatophytoses in regard to the different anatomic sites, age and sex categories was presented. Sex had a significant effect on the prevalence of some clinical presentations ($P < 0.05$), but not on tinea unguium and tinea faciei ($P > 0.05$). Regarding the age distribution, infection occurred in the age range of

1–89 years and the prevalence of infection was the highest in patients aged 21–30. The prevalence of all clinical forms differed significantly in the patients of various age groups ($P < 0.05$) except for tinea unguium manuum.

Primary screening of the isolates based on the macro- and microscopy of the colonies revealed that *T. verrucosum* with 439 cases (42.6 %) was the most frequent agent, followed by *E. floccosum* (366), *T. mentagrophytes* (175), *M. canis* (31), *T. rubrum* (13), *T. violaceum* (3), *T. tonsurans* (2) and *M. gypseum* (2).

On the other hand, amplification and subsequent two-step digestion of the ITS rDNA products led to the identification of *T. interdigitale* as the most frequent isolate (58.7 %), followed by *E. floccosum* (35.4 %), *M. canis* (3 %), *T. rubrum* (1.5 %), *Arthroderma benhamiae* complex (0.5 %), *T. tonsurans* (0.3 %) and *T. violaceum* (0.3 %). Other species with the least frequency included *T. verrucosum*, *M. gypseum* and *M. fulvum* (each one 0.1 %). Sequencing confirmed all identifications made by morphology and RFLP and five isolates which had restriction profile identical to the *A. benhamiae* complex were specified as *T. species of A. benhamiae*. Additionally, ITS1 analysis showed that none of the 72 sequenced *T. interdigitale* isolates were anthropophilic strains (data not shown). All 100 generated sequences in the study were deposited in the GenBank database under accession numbers KT192448 to KT192547 (Table 3).

Discussion

This study, from one side, reasserted the ineffectuality of morphological versus molecular methods for dermatophytes identification and, on the other side, pointed out that epidemiology of dermatophytoses and related causative agents in Iran are evolving [3, 13]. Till now, extensive studies performed on the epidemiological features of dermatophytosis in Iran and the infection has been investigated in most parts of the country by using the traditional culture-based procedures [11, 13–18]. Fortunately, the most recent scrutinizers were propelled to application of reliable molecular methods based on the new species concept of dermatophytes [3, 13]. Currently, the most validated strategy for the precise molecular recognition of dermatophytes is use of the rDNA ITS region for sequence analysis [5] that we applied in our survey.

Table 1 Results of direct examination and culture for diagnosis of infections, and distribution of different clinical manifestations in regard to the sex

Examinations result Form of infection	MpCn	MnCp	MpCp	Total (M/F) ^a	<i>P</i> value ^b
<i>T. faciei</i>	4	1	53	58 (30/28)	.793
<i>T. capitis</i>	15	0	120	135 (103/32)	.000
<i>T. unguium pedum</i>	4	0	42	46 (25/21)	.555
<i>T. unguium manuum</i>	5	0	27	32 (12/20)	.157
<i>T. pedis</i>	8	0	108	116 (75/41)	.002
<i>T. manuum</i>	13	0	113	126 (43/83)	.000
<i>T. corporis</i>	28	2	330	360 (147/213)	.001
<i>T. cruris</i>	15	0	231	246 (164/82)	.000
<i>T. barbae</i>	0	0	4	4 (4/0)	–
Total	92	3	1028	1123 (603/520)	–

^a M/F = male/female^b *P* value from Chi-square test for prevalence of infections between males and females**Table 2** Frequency of different types of infection in association with age categories

Age groups Type of infection	1–10	11–20	21–30	31–40	41–50	>50	Total (%)	<i>P</i> value ^a
<i>T. faciei</i>	27	11	9	3	7	1	58 (5.2)	.000
<i>T. capitis</i>	108	18	5	1	3	0	135 (12)	.000
<i>T. unguium pedum</i>	0	1	6	7	11	21	46 (4)	.000
<i>T. unguium manuum</i>	5	1	8	6	4	8	32 (2.9)	.250
<i>T. pedis</i>	1	4	24	30	33	24	116 (10.3)	.000
<i>T. manuum</i>	4	14	39	30	22	17	126 (11.2)	.000
<i>T. corporis</i>	34	47	120	73	49	37	360 (32)	.000
<i>T. cruris</i>	14	24	104	33	41	30	246 (22)	.000
<i>T. barbae</i>	0	1	3	0	0	0	4 (0.4)	–
Total	193	121	318	183	170	138	1123	–

^a *P* value from Chi-square test between different age groups

In consonance with the majority of national [10, 11, 13, 14, 16–18] and international reports from Brazil, Tunisia, India and Lebanon [19–22], dermatophytoses more occurred in male patients (53.7 %) from Khuzestan. This observation is ascribed to this fact that men are more engaged in prolonged and vigorous outdoor activities in this area of Iran. It is known that the predominant form of dermatophytosis varies widely in different studies [3, 13, 23]. The main clinical entities among our studied patients were tinea corporis and tinea cruris, and two clinical forms, respectively, more occurred in females and males, though their occurrences were highest in the age group of 21–30 years. This model of clinical preponderances has already been addressed [3, 11, 13, 16, 24], and generally, currently the dominant clinical form of dermatophytosis in Middle East is tinea corporis [25]. While, currently, *T. rubrum* is the most widespread agent of tinea cruris worldwide [23], *E. floccosum* is

the predominant causative in Iran [3, 13, 24], the issue that was reaffirmed in our study. Concerning tinea corporis, the human-to-human transmission of *T. rubrum* continues to be the most important mode of spread worldwide [1, 19, 21, 25]. But, this study notified again that nowadays in most parts of Iran tinea corporis is mainly resulted from the zoophilic strains of *T. interdigitale* [3, 13, 26]. *Microsporum* spp. were cited as the dominant agents of tinea corporis in Europe and many other parts of the world [4, 27]. While consistent with the prior studies, our survey showed that *M. canis* and *M. gypseum* cause body infection in Iran with far less frequency [3, 17, 28, 29]. Dermatophytosis of the scalp skin and hairs was for a long time the foremost dermatophytic infection in some parts of the country [14, 15]. Based on recent reports, a decreasing trend in the incidence of tinea capitis was observed nationwide [3, 13, 24]. By contrast, it ranked third in frequency among our

Table 3 Results of dermatophytes identification in the study by ITS-RFLP and sequencing, and their frequency in regard to the clinical form of infections

Type of tinea species by RFLP	<i>T. faciei</i>	<i>T. capitis</i>	<i>T. unguinum</i> (p-m)*	<i>T. pedis</i>	<i>T. manuum</i>	<i>T. corporis</i>	<i>T. cruris</i>	<i>T. barbae</i>	Total (%)	Specie by sequencing (n) (GenBank accession numbers)
<i>T. interdigitale</i>	43	98	28–15	88	86	210	35	3	606 (58.7)	<i>T. interdigitale</i> (72) (KT192467–KT192538)
<i>T. violaceum</i>	0	2	0	0	0	1	0	0	3 (0.3)	<i>T. violaceum</i> (1) (KT192547)
<i>T. rubrum</i>	0	1	5–6	0	0	2	1	0	15 (1.5)	<i>T. rubrum</i> (5) (KT192539–KT192543)
<i>T. tonsurans</i>	0	2	0–0	0	0	1	0	0	3 (0.3)	<i>T. tonsurans</i> (2) (KT192544–KT192545)
<i>T. verrucosum</i>	0	0	0–0	0	0	1	0	0	1 (0.1)	<i>T. verrucosum</i> (1) (KT192546)
<i>A. benhamiae complex</i>	0	1	0–0	0	2	2	0	0	5 (0.5)	<i>T. sp. of A. benhamiae</i> (5) (KT192448–KT192452)
<i>M. canis</i>	1	16	0–0	1	2	8	3	0	31 (3)	<i>M. canis</i> (9) (KT192457–KT192465)
<i>M. gypseum</i>	0	0	0–0	0	0	1	0	0	1 (0.1)	<i>M. gypseum</i> (1) (KT192453)
<i>M. fulvum</i>	1	0	0	0	0	0	0	0	1 (0.1)	<i>M. fulvum</i> (1) (KT192466)
<i>E. floccosum</i>	9	0	9–6	19	23	106	192	1	365 (35.4)	<i>E. floccosum</i> (3) (KT192454–KT192456)
Total	54	120	42–27	108	113	332	231	4	1031 (100)	100

*p = pedum, m = manuum

patients and was limited to ectothrix and endothrix type in manifestations. Given that most episodes of infections (95 %) were from the zoophilic variants of *T. interdigitale* and *M. canis*, contact with feline, canine and domestic animals is the main mode of infection transmission in southwestern Iran. From etiological viewpoint, the dominant agent of tinea capitis is *T. tonsurans* in UK, North America and Mexico, *M. canis* in Australia and many parts of continental Europe and *T. violaceum* in North Africa, China and South Asia [3, 4, 30, 31]. In this study, in the most cases (95 %), the zoophilic variants of *T. interdigitale* and *M. canis* strains overwhelmed the anthropophile species as tinea capitis agents. Such shifts of etiological agents from anthropophiles to zoophiles have recently been reported from Iran and some parts of China by Zhang et al. [3, 13, 24, 30]. However, contrary to the common practice in abroad which pets are becoming the most likely sources of infection, contact with cats, dogs and domestic animals is the main mode of infection transmission in southwestern Iran.

Frequency of dermatophytic infection of the hands in our study [11.2 %] was similar to that obtained by Ansari et al. [26], and most of the affected individuals were from rural areas, and in 80 % of the cases the etiological agents were the *T. interdigitale*, *M. canis* and *T. species of A. benhamiae* species. *E. floccosum* was the only anthropophilic agent involved. This was contrary to investigations from Iran [14, 15] and overseas [19, 20, 32] where *T. verrucosum* and *T. rubrum* were cited as the predominant agent of tinea manuum. The zoophilic dermatophyte *Trichophyton* species of *A. benhamiae* is a new species that is recently derived from the *T. mentagrophytes sensu lato* [1, 5]. It has recently emerged as a new agent of dermatophytosis and nowadays is counted as the most prevalent zoophilic agent of dermatophytosis in Germany [1, 33]. Hitherto, the species has scarcely been cited as agent of infection in Iran [3, 13], not due to its true rarity, but because the species is incorrectly identified as other morphologically similar species. In the most recent report by Ansari et al. [26], 17 clinical isolates of the species were detected from south of Iran.

Dermatophytosis of the feet is known to more impact adult men [13], and consistent with this fact, a significant age and gender relation with prevalence of the infection ($P = .000–.002$) was found among the

studied patients. Contrary to the global trend, in which *T. rubrum* is the main species implicated in tinea pedis [23, 25, 27, 31], and concordant with the latest national assessments [3, 13, 24], *T. interdigitale* remained as the main agent of tinea pedis in Khuzestan. Tinea unguium constituted 6.9 % of all infections in our study, while in some reports from Tunisia, Mexico and Brazil [19, 20, 31] it was the dominant form of tinea. The low prevalence of tinea unguium is attributed to a shift from dermatophyte toward non-dermatophyte molds and yeasts nail infection [34, 35]. Generally, nail invasion by dermatophytes is more common in male and elderly people [36]; however, in our study the gender distribution of infection was not meaningful and in view of age distribution only toenail infection was significant ($P = .000$). Moreover, as a national and universal trend, onychomycosis more tends to be caused by *Trichophyton* spp., than *Microsporum* spp., and *T. rubrum*, *T. interdigitale* and *E. floccosum* are the main species implicated in the infection [3, 13, 23, 27]. In line with this expectation, nail infection in our patients was exclusively resulted from the three mentioned species.

Concerning tinea faciei, fewer or no attentions have been paid to the infection in previous studies [3, 14]. In our study, it was significantly more manifested in the age category of 1–10 years and due to *T. interdigitale* species. Totally, dermatophytosis of the non-bearded areas of the face is a rare infection that is known to occur more often among children and women due to close contact with pets [3, 32]. But, pets are obviously less beloved in Islamic states, and the possible sources of this infection are either infected individuals or domesticated animals [3]. Consistent with this fact, *T. interdigitale* (95 %), *M. canis* and *M. fulvum* (each one 2.5 %) were the species corresponding to tinea faciei among Khuzestanian patients.

Infection of the beard and mustache accounted for the least frequent type of infection in the study (0.4 %), and *T. interdigitale* was the main causative agent. Generally, tinea barbae was more common in the past before single-use razors became available, but recently took a decreasing trend in Iran and currently it is infrequent around the world [3, 13, 24].

Concerning the causal agents, *T. interdigitale sensu stricto* (58.7 %) appeared as the main agent of all clinical presentations (except tinea cruris), which corroborated the recent findings of molecular-based surveys from Tehran, the capital, and south of Iran

[3, 13, 24, 26]. The dominant agent of dermatophytoses tends to vary in each study, but the current high occurrence of infection with *T. interdigitale* can be attributed to an adaptation between identification methods and the new species concept of dermatophytes [5]. The recent sequence-based scrutinizes in Iran together with this survey clearly demonstrated that most of the isolates morphologically masqueraded as *T. verrucosum* are, indeed, a more diverse range of fungal entities including *T. interdigitale sensu stricto*, the zoophilic dermatophyte *T. species of A. benhamiae* and *T. verrucosum* [3, 13, 26]. Similarly, most of the isolates that traditionally characterized as *T. mentagrophytes* (also *T. mentagrophytes* complex) were found to be a diverse taxonomic group including *T. interdigitale* and *A. benhamiae* species. According to the biological and molecular findings, today it is known that *T. mentagrophytes sensu stricto* is synonymous only with the zoophilic variants and species that formerly recognized as *T. mentagrophytes* var. *quinckeanum*, *T. langeronii* and *T. sarkisovii* [5, 37], and thus, the real prevalence and ecology of this zoophilic species in Iran is unknown. Low frequency of infection with *T. rubrum* in Khuzestan was similar to the recent study by Naseri et al. [18], and while an apparent decrease in the rate of infection with anthropophilic species was observed, *E. floccosum* remained the main human-adapted species causing dermatophytosis in southwestern Iran.

Overall, findings of the current survey signaled that distribution patterns of dermatophytes have notably changed in southwestern Iran. *Trichophyton interdigitale*, especially the zoophilic variant, and *T. species of A. benhamiae* have, respectively, appeared as the dominant and new emerging agents of dermatophytosis in this area of Iran.

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Compliance with Ethical Standards

Conflict of interest None.

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