Short Communications

Molecular epidemiology of dermatophytosis in Tehran, Iran, a clinical and microbial survey

ALI REZAEI-MATEHKOLAEI*, KOICHI MAKIMURA†, SYBREN DE HOOG‡, MOHAMMAD REZA SHIDFAR§, FARIDEH ZAINI§, MOHAMMADREZA ESHRAGHIAN#, PARVANEH ADIMI NAGHAN§ & HOSSEIN MIRHENDI§

*Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
†Teikyo University, Institute of Medical Mycology, Tokyo, Japan
‡Fungal Biodiversity Center, Institute of the Royal Netherlands Academy of Arts and Sciences, CBS-KNAW, Utrecht, The Netherlands, and Departments of §Medical Parasitology & Mycology and #Biostatistics and Epidemiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

In the framework of a survey on dermatophytoses, 14,619 clinical specimens taken from outpatients with symptoms suggestive of tinea and referred to a Medical Mycology laboratory in Tehran, Iran, were analyzed by direct microscopy and culture. In total, 777 dermatophyte strains recovered in culture were randomly identified by a formerly established RFLP analysis method based on the rDNA ITS regions. For confirmation of species identification, 160 isolates representing the likely entire species spectrum were subjected to ITS-sequencing. Infection was confirmed in 5,175 collected samples (35.4%) by direct microscopy and/or culture. Tinea pedis was the most prevalent type of infection (43.4%), followed by tinea unguium (21.3%), tinea cruris (20.7%), tinea corporis (9.4%), tinea manuum (4.2%), tinea capitis (0.8%) and tinea faciei (0.2%). Trichophyton interdigitale was the most common isolate (40.5%) followed by T. rubrum (34.75%), Epidermophyton floccosum (15.6%), Microsporum canis (3.9%), T. tonsurans (3.5%) and M. gypseum (0.5%). Other species included M. ferrugineum, T. erinacei, T. violaceum, T. schoenleinii, and a very rare species T. eriotrephon (each one 0.25%). The two strains of T. eriotrephon isolated from tinea manuum and tinea faciei are the second and third reported cases worldwide. Application of DNA-based methods is an important aid in monitoring trends in dermatophytosis in the community.

Keywords dermatophytosis, molecular epidemiology, ITS-PCR-RFLP, Tehran

Introduction

Dermatophytes are a large group of fungi affecting the keratinized tissues (skin, hair and nails) in humans and animals, causing a spectrum of infections medically known as dermatophytosis [1]. Infections may be transmitted from the environment, from domesticated animals or from humans, and remain a public health concern in all communities [2]. Many non-infectious dermatological disorders resemble superficial fungal infections should be included in differential diagnoses of dermatophytosis [3]. Epidemiological profiles of dermatophytes and dermatophytes tend to alter over time due to climatic, environmental, or socio-economic factors, and can also be influenced by tourism [4,5]. Additionally, precise identification of etiologic agents from suspected lesions is important for appropriate therapy and control of potential environmental sources of infection [3,6].

In this study we randomly selected 777 dermatophyte strains isolated from patients in Tehran, Iran, and used PCR-restriction fragment length polymorphism (RFLP) to identify them. A significant number of isolates were also subjected to ITS-sequencing to reconfirm their identity at
the species level to obtain reliable epidemiological data. This is the first large-scale report on molecular identification/epidemiology of dermatophytes in Iran.

**Material and methods**

A total of 14,619 clinical specimens (nail clipping, skin scrapings, scalp scales and hair) were obtained from lesions of outpatients suspected of dermatophytosis referred to a medical mycology laboratory in Tehran during 2008–2010. Routinely, one part of each sample was predigested with 20% potassium hydroxide and examined microscopically, and another portion was inoculated onto Mycobiotic agar (Difco, USA). Macroscopic and microscopic characteristics were studied and non-dermatophyte molds were excluded. For molecular identification, the following reference strains were used as controls:

- *T. interdigitale*: NBRC (NITE Biological Resource Center) 5974, NBRC 5809, NBRC 5466 and NBRC 5812; *Arthroderma vanbreuseghemii*: JCM (Japan Collection of Microorganisms) 1891; *A. benhamiae*: JCM 1885; *M. persicolor*: NBRC 5975; *T. tonsurans*: NBRC 5928 and NBRC 5945; *T. equinum*: NBRC 31610; *T. rubrum*: NBRC 5808 and NBRC 5467; *T. violaceum*: NBRC 31064; *Epidermophyton floccosum*: NBRC 9045; *Microsporum canis*: NBRC 9182; *M. ferrugineum*: NBRC 6081 and NBRC 5831; *M. audouini*: NBRC 6074; *A. obtusum*: JCM 1907; *A. uncinatum*: NBRC 31978; *T. schoenleinii*: NBRC 8192 and NBRC 8191; *M. cookei*: NBRC 7862; *M. gypseum*: NBRC 8228 and NBRC 5948.

DNA was prepared from cultures using the method described by Makimura et al. [7], with the rDNA ITS regions of reference strains and 777 randomly selected clinical isolates amplified using primers ITS1 and ITS4 [8]. In the preliminary screening, all amplicons were subjected to digestion with the restriction enzyme *MvaI*, as per a previously described RFLP assay [9]. For identification of strains that had identical RFLP pattern to *T. tonsurans/T. equinum* or *M. canis/M. ferrugineum*, two additional RFLP assays with *BsrFI* and *MaelIII*, respectively, were carried out as described previously [10,11]. Figure 1 illustrates the summary of molecular tests performed in the study.

Amplified and digested products were electrophoresed on agarose gel and identification of the isolates was carried out through comparing the electrophoretic RFLP patterns with those profiles obtained previously [9–11] and summarized in Figure 1. For identification we considered the most recent classification for dermatophyte species [12]. To confirm the consistency of the three PCR-RFLP assays and to provide molecular data of Iranian strains, ITS regions of 160 clinical isolates representing all species were subjected to bidirectional sequencing with ITS1/ITS4 primers as described previously [9].

### Results

Infection was confirmed by direct examination and/or culture in 5,175 (35.4%) of the 14,619 clinical specimens (Table 1). Among these, 949 (18.3%) samples were found to be positive through the use of only direct microscopy, 4,167 (80.5%) by both microscopy and culture, and 59 (1.2%) only by recovery of the fungus in culture. The patients from whom samples were collected included 2,893 (55.9%) males and 2,282 (44.1%) female.

**Fig. 1** The flowchart of the molecular identification of clinical isolates of dermatophytes in this study. (Numbers in parenthesis are fragments size of RFLP products for each relevant restriction enzyme.)
Frequencies and relationships of clinical forms and causative species are summarized in Table 2. Tinea pedis was the predominant clinical manifestation, followed by tinea unguium, tinea cruris, tinea corporis, tinea manuum, tinea capitis and tinea faciei. In primary screening performed by ITS-RFLP with MvaI, the generated profiles of 714 out of 777 isolates matched with expected specific patterns as previously reported [9], leading to identification of T. interdigitale (n = 315), T. rubrum (n = 270), E. floccosum (n = 121), M. gypseum (n = 4), T. schoenleinii (n = 2) and T. violaceum (n = 2). In a second ITS-RFLP assay of 27 isolates that had MvaI RFLP profiles consistent with either T. tonsurans or T. equinum, all strains were identified as T. tonsurans through the use of BsrFI. Among 32 strains that had MvaI RFLP patterns of either M. canis or M. ferrugineum, 30 strains were identified as M. canis and two as M. ferrugineum by the second digestion with MaeIII. For identification of four remaining isolates that had MvaI RFLP profiles specific for the Arthroderma benhamiae complex [9], ITS-sequencing identified two isolates as T. erinacei and two as T. eriotrephon. The results of ITS-sequencing from all representative strains were deposited in GenBank as accession numbers JN133969 to JN134156. Table 2 compares the results of dermatophyte identification with ITS-RFLP and ITS-sequencing for 160 representative samples.

**Discussion**

Correct identification of etiologic agents is important to provide a baseline for epidemiologic studies, to elucidate changes in frequency, to evaluate interventions, and to

---

**Table 1** The results of direct microscopy and culture for detection of dermatophytosis in the study.

<table>
<thead>
<tr>
<th>Tests</th>
<th>DnCn</th>
<th>DpCp</th>
<th>DpCn</th>
<th>DnCp</th>
<th>Total specimens (%)</th>
<th>Total positive specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinea cruris</td>
<td>1440</td>
<td>918</td>
<td>150</td>
<td>2</td>
<td>2510 (17.2)</td>
<td>1070 (20.7)</td>
</tr>
<tr>
<td>Tinea unguium</td>
<td>3282</td>
<td>822</td>
<td>258</td>
<td>23</td>
<td>4385 (30)</td>
<td>1103 (21.3)</td>
</tr>
<tr>
<td>Tinea pedis</td>
<td>1836</td>
<td>1807</td>
<td>414</td>
<td>27</td>
<td>4084 (27.9)</td>
<td>2248 (43.4)</td>
</tr>
<tr>
<td>Tinea manuum</td>
<td>213</td>
<td>168</td>
<td>48</td>
<td>0</td>
<td>429 (2.9)</td>
<td>216 (4.2)</td>
</tr>
<tr>
<td>Tinea corporis</td>
<td>2216</td>
<td>404</td>
<td>76</td>
<td>5</td>
<td>2701 (18.5)</td>
<td>485 (9.4)</td>
</tr>
<tr>
<td>Tinea capitis</td>
<td>427</td>
<td>39</td>
<td>3</td>
<td>0</td>
<td>469 (3.2)</td>
<td>42 (0.8)</td>
</tr>
<tr>
<td>Tinea faciei</td>
<td>30</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>41 (0.3)</td>
<td>11 (0.2)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>9444 (64.6)</td>
<td>4167 (28.5)</td>
<td>949 (6.5)</td>
<td>59 (0.4)</td>
<td>14,619 (100)</td>
<td>5,175 (35.4)</td>
</tr>
</tbody>
</table>

DnCn, Direct microscopy negative and Culture for dermatophytes negative; DpCp, Direct microscopy positive and Culture for dermatophytes positive; DpCn, Direct microscopy positive and Culture for dermatophytes negative; DnCp, Direct microscopy negative and Culture for dermatophytes positive.

**Table 2** Frequencies of dermatophyte species isolated in the study and their distribution according to the clinical forms of dermatophytosis; and comparison between ITS-RFLP and ITS-sequencing for identification of representative dermatophyte isolates.

<table>
<thead>
<tr>
<th>Species identification by PCR–RFLP</th>
<th>Clinical forms</th>
<th>Species identification by ITS-sequencing (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichophyton interdigitale</strong></td>
<td>Tinea pedis</td>
<td>T. interdigitale (56) (JN133969 – JN134024)</td>
</tr>
<tr>
<td>Arthroderma benhamiae complex</td>
<td>Tinea corporis</td>
<td>T. erinacei (2) (JN134088, JN134091)</td>
</tr>
<tr>
<td>A. benhamiae complex</td>
<td>Tinea cruris</td>
<td>T. eriotrephon (2) (JN134089 – JN134090)</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>Tinea faciei</td>
<td>T. rubrum (42) (JN134025 – JN134066)</td>
</tr>
<tr>
<td>Epidermophyton floccosum</td>
<td>Tinea unguium</td>
<td>E. floccosum (11) (JN134146 – JN134156)</td>
</tr>
<tr>
<td>Microsporum canis</td>
<td></td>
<td>M. canis (21) (JN134109 – JN134128)</td>
</tr>
<tr>
<td>M. gypseum</td>
<td></td>
<td>M. gypseum (3) (JN134069 – JN134085)</td>
</tr>
<tr>
<td>M. ferrugineum</td>
<td></td>
<td>M. ferrugineum (2) (JN134135 – JN134136)</td>
</tr>
<tr>
<td>T. schoenleinii</td>
<td></td>
<td>T. schoenleinii (2) (JN134096 – JN134097)</td>
</tr>
<tr>
<td>T. violaceum</td>
<td></td>
<td>T. violaceum (2) (JN134104 – JN134105)</td>
</tr>
<tr>
<td>Total (n)</td>
<td></td>
<td>160</td>
</tr>
</tbody>
</table>

*The accession numbers are related to the isolates of this study.

© 2013 ISHAM, Medical Mycology, 51, 203–207
reveal new pathogens [13]. Studies have proven that the DNA-based assays targeting the rDNA ITS regions provide reliable screening tools for these purposes [9–11,14]. In this study we presented an extended survey on the distribution of dermatophytosis, with major emphasis on their molecular identification in a single mycology laboratory in Tehran, Iran. Distribution of dermatophytes was relatively high in our analysis, indicating that the infection they cause still remains as a health problem in Tehran.

The most frequently observed manifestations were tinea pedis, tinea unguium, tinea cruris and tinea corporis. The distribution of tinea pedis in males was more than females. The main species recovered from foot infections were *T. interdigitale*, *T. rubrum* and *E. floccosum*, with *T. rubrum* being responsible for the most cases worldwide [4,5]. Tinea unguium was the second most commonly encountered clinical form in our study and *T. rubrum* (64%), *T. interdigitale* (32%) and *E. floccosum* (4%) were the species implicated in these infections. *E. floccosum*, *T. rubrum* and *T. interdigitale* were the main agents of tinea cruris in our samples and are known to be the primary cause of this clinical form in other studies [5,6]. Noteworthy, there was a case of groin infection by *T. erinacei* in our samples, which is the first confirmed case of tinea cruris by this species in Iran. *T. erinacei* is a zoophilic dermatophyte that recently was segregated from the former *T. mentagrophytes* complex and received species status [15]. We used sequencing for final delineation of the isolates in the *A. benhamiae* complex. It is likely that if molecular methods are applied more widely in epidemiologic studies, the report of such less common species may increase.

Ringworm of the body ranked fourth in frequency and *T. interdigitale* was the most frequent etiologic agent, followed by *T. rubrum*, *M. canis*, *T. tonsurans* and *E. floccosum*, whereas *T. violaceum* and *M. gypseum* were the least frequent causes of this infection. Our findings are contradictory to other studies in Iran where *T. tonsurans* and *T. verrucosum* [6] were the dominant causes of tinea corporis. Infections of the hands, head (scalp and hair) and face were much lower in frequency, all together accounting for 5.2% of total infections. Most common causative agents were *T. interdigitale* (*n = 7*) and *T. rubrum* (*n = 5*), while *T. erinacei*, *T. tonsurans*, *M. gypseum* and *E. floccosum* were each isolated once. Similar data on the etiology of tinea manuum were reported from other studies conducted in Iran, although the dominant agent tended to vary with each study [6,16]. The strain of *T. erinacei*, isolated from a hand infection, is the first proven case of tinea manuum by this species in Iran. The frequency of tinea capitis was low (2.1%) in the present investigation. Ectothrix was the main pathological type of scalp and hair infection followed by endothrix and favus. The recent decrease in the prevalence of tinea capitis in Asiatic countries is probably due to an improvement of sanitary and socioeconomic conditions [4]. At present the most common causes of tinea capitis worldwide are *T. tonsurans*, *M. canis*, *M. audouinii*, *T. violaceum*, *T. schoenleinii* and *M. ferrugineum*; nevertheless, the species distribution differs from one region to another [4,5]. In the current study, the causative agents of infection in decreasing order of frequency were *M. canis*, *T. tonsurans*, *T. schoenleinii*, and *T. interdigitale*, while *M. gypseum*, *M. ferrugineum* and *T. eriotrephon* were each recovered only once.

The most interesting aspect of the present study might be the isolation of two strains of a very rare dermatophyte, *T. eriotrephon*. The only strain known of this species was isolated in 1925 from a patient affected by tinea corporis in The Netherlands [17]. In this study we identified two new cases due to this species in Iranian patients affected by tinea capitis and tinea faciei. In the preliminary screening by ITS-RFLP with *MvaI* both strains were suspected as *A. benhamiae* complex, but ITS-sequencing enabled us to identify them as *T. eriotrephon*. The recent study on ITS regions indicated that this species is closely related to the Americano-European race of *A. benhamiae* [15]. Our strains were the second and third report of this rare species worldwide, but the species is likely to be reported more frequently by application of molecular approaches.

Tinea faciei was the least frequent clinical form in our study with only 11 cases. The infection occurs more often in children due to contact with pets such as dogs, cats and rabbits transmitting zoophilic species, like *M. canis* or zoophilic strains of *T. interdigitale* [18,19]. In our analysis, most of the infected individuals were younger than 10 years (data not shown) and the etiologic organisms involved were *T. tonsurans*, *M. canis*, *T. eriotrephon* and *T. interdigitale*. Apparently pet animals are less popular in Iran and infection sources are either domestic animals or human sources. The predominance of the anthropophilic species *T. tonsurans* (*n = 5*) among culture isolated agents of tinea faciei in the study is in line with this claim.

We did not observe any case of tinea barbae in the current study. This was expected since nowadays men pay more attention to their personal hygiene and this infection recently has shown decreasing trends in Iran [6]. Although *T. verrucosum* constituted a significant portion of isolates in previous studies [6,20,21], surprisingly no case involving this species was recognized in the present study. Some discrepancy may have been caused by phenotypic versus molecular identification methods. For example, all 17 strains which we morphologically identified as *T. verrucosum* were in fact *T. interdigitale* when subjected to both PCR-RFLP and sequencing, in accordance with recent changes in the taxonomy of dermatophyte species [12,22]. The strains identified as *T. verrucosum* var. *autotrophicum* have recently been considered to be conspecific with
A. vanbreuseghemii (T. interdigitale) based on molecular data [12,22].

In conclusion, although there are not enough historical studies about the frequency of dermatophytes and dermatophytoses in Iran, when our data are compared to previous investigations it seems that the proportions of zoophilic and geophilic dermatophyte caused infections are decreasing, while those due to anthropophilic species have increased. Utilization of DNA-based identification methods such as sequencing or PCR-RFLP that are more reliable than classical phenotypic methods was an important strength of our survey, as proven by the detection of a rare species as T. eriotrephon.

Acknowledgements

This work was financially supported by both Tehran University of Medical Sciences (TUMS, Grant no: 87-01-27-6962), Tehran, Iran; and Teikyo University Institute of Medical Mycology (TIMM), Tokyo, Japan. We thank all personnel in Molecular Biology Laboratory in TIMM and in Medical Mycology laboratory in TUMS.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References


This paper was first published online on Early Online on 18 May 2012.