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ORIGINAL ARTICLE

Molecular study and antifungal susceptibility profile of *Trichophyton rubrum* and *Trichophyton mentagrophytes* strains isolated from lesions of humans and cattle

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ABSTRACT

Background and Objectives: Monitoring of contagious diseases is important to advance our knowledge of their epidemiology and to enable more impressive investigation and prevention efforts. This study aimed to examine antifungal drug susceptibility and molecular analysis of clinical isolates of *Trichophyton rubrum* and *Trichophyton mentagrophytes* in humans and cattle.

Materials and Methods: A total of 400 patients and 500 cattle were evaluated in this study. Dermatophytosis was confirmed in cases by direct microscopy and culture methods. Antifungal drug susceptibility profiles, MIC_{50}, and MIC_{90} of isolates were determined using the broth microdilution method. Multiplex-PCR, RAPD PCR, and sequencing methods were used for the genetic analysis of virulence genes and the ITS1 and ITS2 regions, respectively.

Results: A total of 175 patients and 120 cattle were diagnosed with dermatophytosis. Dermatophytes showed a remarkable rate (30%) of terbinafine resistance. *T. mentagrophytes* showed lower susceptibility than *T. rubrum* (MIC_{50}=16 µg/mL). Strains harboring *Mep1*, *Mep2*, and *Mep4* genes had the highest frequency among all genotypes. A RAPD-PCR dendrogram divided *T. mentagrophytes* and *T. rubrum* strains into three and six groups, respectively.

Conclusion: A notable rate of resistance to terbinafine in isolated dermatophytes was reported in this study. Examination of RAPD-PCR results showed that *T. rubrum* strains had higher genetic diversity than *T. mentagrophytes*. Genetic monitoring of dermatophytes must be considered an important factor in providing fungal infection prevention and treatment approaches.

Keywords: *Trichophyton rubrum*; *Trichophyton mentagrophytes*; Dermatophytosis; Random amplified polymorphic DNA-PCR

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INTRODUCTION

Dermatophytes are closely related to the homogeneous clade of keratinophilic filamentous fungi and the most important and abundant fungal agents of the skin that attack the skin, nails, and hair. This group of fungi are responsible for many superficial fungal infections in humans and animals. In infection by this group of fungi, there are three stages of attachment to the host tissue, attack, and expansion of the host immune response, which use special enzymes in the attack stage of the host (1). Dermatophytosis, also called ringworm, is the most common infectious disease among the ten major isolated human pathogens in tropical and subtropical countries, including Iran. The common causes of the disease are *Trichophyton rubrum (T. rubrum), Trichophyton mentagrophytes (T. mentagrophytes),* and *Microsporum canis* (2).

These microorganisms require keratin for growth, nonetheless, they can survive on keratin-free surfaces; in a superficial infection, however, the production and release of proteolytic enzymes allow the fungus to penetrate the host tissue and survive. The severity of the disease depends on the dermatophyte species, susceptibility, and general conditions of the host (3).

Dermatophytosis is usually regarded as an unimportant health issue for human and animals since a wide range of antifungal drugs is available. In recent times, however, epidemics by several dermatophyte species have been reported around the world with considerable virulence and resistance to commonly applied antifungals (2, 3).

T. rubrum is the most frequent causative agent of human dermatophytosis that can escape or suppress the host immune system during the infection process and, unlike other fungi, can cause infections in healthy, immune-competent people. The microorganism is specialized to infect humans, but there are some studies reporting animal infections. *T. mentagrophytes* is also a major cause of dermatophyte infection in humans and animals, especially cattle and sheep (4).

Previous studies have reported that many genes are involved in resistance to antifungal drugs and the pathogenesis of the dermatophytes. Metalloproteases of the M36 family is one of the vital virulence factors for skin invasion by dermatophytes that are encoded by the MEP genes and demonstrate strong proteolytic and hair biodegradation activity. In addition, exoproteases (e.g., NPII, carboxypeptidase S1 homolog A and B, ScpA, B) play a significant role in the degradation of skin hard keratin. These genes are involved in production of extensive array of endo- and exoproteases as virulence determinants that facilitates the keratin hydrolysis. Ergosterol is an important component of the cell membranes of fungal microorganisms that are encoded by the ERG gene family and is the main target of azole drugs. Interruption of ergosterol biosynthesis in dermatophytes results in drug resistance (5).

Diagnosis of infections caused by Trichophytons is very difficult and can delay treatment for months. Therefore, rapid diagnostic tests using precise molecular methodologies have attracted considerable attention (6). In addition, rising resistance to antifungal drugs is another important problem in clinical treatments. Systemic antifungal drugs are prescribed for severe dermatophyte infections, including nails, scalp, and feet, which do not respond to topical treatment alone (7). However, it has been shown that therapies have a low success rate, and resistance to antifungal drugs has eliminated available medicine classes as treatment choices. New understandings of genetic factors regulating this antifungal resistance and fungal pathogenesis mechanisms provide a foundation for new therapeutic strategies (4, 8).

Now, there are few drugs permitted for the handling of invasive mycoses, and the efficacy of these medications is compromised by the development of drug resistance in Trichophytons (8). Identifying the susceptibility pattern of antifungal drugs, the genomic characteristics of pathogenicity factors, and their distribution in different species of dermatophytes leads to a better understanding of the process of dermatophytosis to provide effective prevention strategies (7). Though the internal transcribed spacer (ITS) sequencing is informative, it has been approved that dermatophyte fungi, especially trichophytons, are a closely related group, but it remains challenging to use this marker to discriminate isolates amongst species complexes (9). In addition, there is rare information about the genomic characteristics including frequency of polymorphism in virulence genes and the antifungal drug susceptibility pattern of dermatophytes in the Iranian population. Given this background, we studied clinically isolated *T. rubrum* and *T. mentagrophytes* genetic characteristics and antifungal drug resistance profile.

MATERIALS AND METHODS

Sampling. Between May 2020 and May 2021, 532 samples were collected from 400 patients and 120 cattle suspected of dermatophytosis. Signed written consent was obtained from each participant for using their specimens in this study. Demographic information of individuals was used for epidemiological study. Cattle were examined in terms of dermatophytosis and diagnosed with lesions such as crusts, bleeding, alopecia, scaling, hair emaciation, dullness, itching, anatomical location of lesions, and age, followed by recording the onset of disease symptoms. The samples included specimens of skin, hair, and different parts of the body that were collected by five trained specialists in dermatology and veterinary. Skin scrapings were collected in a sterile condition. Firstly, cotton wool soaked in 70% alcohol was used to remove lesion site surface adhering microorganisms, and the edges of the lesions were scraped using a sterile scalpel blade into the sterile packet.

Isolation and identification of *T. rubrum* and *T. mentagrophytes*. Samples were microscopically evaluated using 20% potassium hydroxide (KOH) with 40% Dimethyl sulphoxide (DMSO) solutions. Slides were carefully examined under low-power 10× and 40× magnification for the presence of hyphae and/or arthroconidia. Next, specimens were inoculated in Sabouraud dextrose agar (SDA) containing 16 µg/ml of chloramphenicol and 0.5 mg/ml of cycloheximide (SCC) and incubated aerobically at 28°C for 4 weeks (10). The fungal infection agents were identified based on the gross morphology of the fungal colony and microscopic characterization of their accessory structures and conidia including shape, size, and type of macroconidia. Red pigment production indicated the growth of *T. rubrum* colony on SCC or the specific Dermatophyte Test Medium (DTM). The culture method complemented the direct method.

Antifungal drug susceptibility test. Antifungal drug susceptibility profile was determined using the CLSI M27-A4 broth two-dimensional (eight-by-twelve) checkerboard microdilution method (11). Briefly, stock solutions of antifungal drugs, including terbinafine (TER), griseofulvin (GRI), itraconazole (ITC), luliconazole (LUL), lanoconazole (LAN), ketoconazole (KTC), butenafine (BUT), and econazole (ECO) (Cipla, India), were prepared in DMSO (Sigma, USA) and diluted in Roswell Park Memorial Institute (RPMI) 1640 Medium buffered to pH 7.0 with L-glutamine without bicarbonate. All isolated *T. rubrum* and *T. mentagrophytes* strains were exposed to different concentrations of antifungal drugs in 96-well microplates, incubated at 35°C, and visually evaluated for the growth of dermatophytes after 48 and 72 h. Furthermore, the minimum inhibitory concentration (MIC) at which 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates were inhibited was calculated here. The MIC was defined as the point at which the growth of dermatophytes was inhibited by 80% for antifungals in comparison with the control (*Trichophyton rubrum* PTCC 5143 and *T. mentagrophytes* PTCC 5054). All tests were performed in triplicate.

Multiplex PCR. The genomic DNA of the dermatophytes was extracted using the Qiagen DNeasy Plant Tissue Kit (QIAGEN, Germany). Following quality confirmation by Nanodrop (Eppendorf, Germany), specific primers for target genes were designed by the Gene runner software and blasted on the NCBI website to confirm specificity. Multiplex PCR was used to detect isolates harboring *Mep1-5, Erg11, 24, 26, ScpA, B,* and *NPII* genes. The reaction mixture of PCR amplification was adjusted to 50 µl, which included 50 ng of genomic DNA solution, 10× PCR buffer, 0.6 U of *Taq* polymerase (Merck, Germany), 0.1 mM of dNTPs, and 0.5 mM of the primer (Table 1). The temperature steps of the PCR reaction were performed as follows: initial denaturation step at 95°C for 5 min in 35 cycles, including 95°C denaturation for 30 sec, and primer binding at 56°C for 30 sec. The amplification step was performed at 72°C for 1 min; after 35 cycles, the final amplification step was carried out at 72°C for 10 min. PCR products were electrophoresed on 1.5% agarose gel in the presence of positive and negative controls, stained with erythrogel, and photographed by a gel documentation device.

Random amplification of polymorphic DNA PCR. The sequences of the ITS regions of ribosomal DNA in the isolates were evaluated for further identification of dermatophytes. The primer pairs used for (random amplification of polymorphic DNA) RAPD-PCR were (5'-d[GGTGCGGGAA]-3') and (5'-d[GTTTCGCTCC]-3') (12). The PCR was performed in volumes of 25 mL containing 50 ng of template DNA, a lyophilized mixture of a reaction buffer [25 mM of KCl, 3 mM of $MgCl_2$,and 15 mM of Tris

Table 1. The primers used for the detection of genes and the amplicon size of this gene

Primer	Sequence (5′ to 3′)	Amplicon (bp)
Mep1	F: GCCACTGAGCTGGTTAAG	1950
	R: CTTTGGATCGAACTTAGC	
Mep2	F: AGAGTTCCTGACTCGGAC	1464
	R: ACTCGTGGATGACAATACC	
Mep3	F: GCCATGTCCTTCTCCAAG	2000
	R: GCCATGTCCTTCTCCAAG	
Mep4	F: ATCGTGATTCCTTTAGCACC	257
	R: TCGCCCATGGTATAGTCAG	
Mep5	F: CCAGCTACATGAGTTCAGATG	1648
	R: ACAGGATGTGTAGACCAATGG	
Erg11	F: ACCGCCTTTAGTCTTCCA	213
	R: GTTGCCCTTGATTCCCA	
Erg24	F: GCCAACATTGTGATAGCCT	112
	R: TATTTCCACGGATAGCGA	
Erg26	F: GCCAACATTGTGATAGCCT	72
	R: TATTTCCACGGATAGCGA	
NPII	F: GATGGTAAGGAGATCCAGTTC	920
	R: TTAGCAGCCAACGTAGATAGC	
ScpA	F: GTTGTCGACTTCAAGGCTTCCCTCCACCCGTT	1973
	R: CTTGTCGACGCGGCCGCCTACAAGAAGAAAGCAAG	
ScpB	F: CTTCTCGAGCTCAGTTCCCACCAAAACCGG	2045
	R: CTTGGATCCTTACATTGCCAGCTCTATAAC	

(pH= 8.3)], 0.4 mM of each dNTP, BSA 2.5 mg, 25 pmol of primer, and thermostable polymerases with Bio-Rad thermal cycler T100. The PCR conditions were as follows: denaturation at 95°C for 3 min, 35 cycles of 30 s at 95°C, 30s at 42°C, and 90s at 72°C, and a final extension step at 72°C for 10 min. PCR products were separated by electrophoresis on 2% agarose gel, visualized by staining with ethidium bromide, and photographed under UV. The desired strains were sequenced by Bioneer Company (South Korea) using general primers of ITS1 and ITS-2 regions (13) as shown in Table 2.

To investigate the genomic similarity of *T. rubrum* and *T. mentagrophytes* strains, a dendrogram was drawn using the Numerical Taxonomy and Multivariate Analysis System *(NTSYS-pc)* software plus the Unweighted Pair Group method with the Arithmetic Mean (UPGMA) method. The efficiency of these primers in genotyping was evaluated using the Simpson Coefficient.

Table 2. The primers of ITS1 and ITS-2 regions used for sequencing the desired strains.

Primer	Sequence (5′ to 3′)
ITS-1-F	TCCGTAGGTGAACCTGCGG
ITS-1-R	GCTGCGTTCTTCATCGATGC
ITS-2-F	GCATCGATGAAGAACGCAGC
ITS-2-R	TCCTCCGCTTATTGATATGC

Statistical analysis. The SPSS-18 software was used for statistical analysis and comparison of the results by Excel 2016 statistical software to further analyze the data. Moreover, qualitative data were analyzed using $\chi 2$ *(Chi-square test)* and the Fisher-Mann-Whitney U test. Correlations were calculated and the means were compared by t-test with a significance level of $P < 0.05$.

RESULTS

Identification of dermatophytes. The patients consisted of 200 males and 200 females with an average age of 29 years (range 24-70 years). A total of 175 cases of dermatophytosis were diagnosed in 400 patients (43.75%) clinically suspected of ringworm. The patient's clinical features are summarized in Fig. 1.

Identification results based on biochemical tests. Fifty-four strains of *T. mentagrophytes* (n=33) and *T. rubrum* (n=21) were isolated from patients and cattle. Eight species of dermatophytes were identified in the patient's evaluation. The most frequent causative fungi of dermatophytosis in participants were *Epidermophyton floccosum* followed by *Trichophyton verrucosum, T. rubrum, T. mentagrophytes, Microsporum canis, M. gypseum, Trichophyton tonsurans,* and *T. schoenleinii* (Fig. 2). *T. mentagrophytes* and *T. rubrum* were isolated from the nail, hair, and body, while *T. verrucosum* from bear and *E. floccosum* were isolated from groin and foot. Generally, 24 strains of *T. mentagrophytes* (n=12) and *T. rubrum* (n=12) were isolated from patients.

The isolated fungi from cattle were 57 strains of *T. verrucosum,* 21 strains of *T. mentagrophytes,* 9 strains of *T. rubrum,* 11 strains of *Mucor,* 10 strains of *Lichtheimia,* and 10 strains of *Fusarium.*

Antifungal drug susceptibility. The susceptibility patterns obtained from antifungal susceptibility tests of 54 clinically isolated *T. rubrum* and *T. mentagrophytes* complex strains are detailed in Table 3. For both dermatophytes, the in vitro values of TER were generally lower than the other antifungal agents ($p<0.001$). MIC determination of antifungal drugs against animal isolated strains is showed in Table 4.

Molecular analysis. The results of the presence of the studied virulence genes in *T. rubrum* and *T. mentagrophytes* strains are shown in Table 4. RAPD-PCR results for *T. rubrum* and *T. mentagrophytes* strains are shown in Fig. 3. The dendrogram was drawn with the proper cut-off number (Figs. 3 and 4).

The results of Chi-square analysis showed the significant presence of *Erg11* and *Erg24* genes in *T. mentagrophytes* strains ($P\leq0.001$). There was a significant difference in the *NPII* gene presence frequency according to RAPD-PCR ($P\leq0.002$) in the mentioned isolates. The existence frequency of the *Erg24, Mep1,* and *Mep 2* genes was significant ($P\leq0.014$, $P\leq0.013$, and $P\leq0.0001$, respectively) in *T. rubrum.* The Simpson coefficient method showed a significant correlation between the presence of *Mep1/Mep2* and *Mep5* genes ($p = 0.000$) in *T. rubrum* strains. This correlation was also statistically significant between the presence of *ScpA* and *Mep1/Mep2* genes ($p=0.000$) (Table 5).

DISCUSSION

In our study the most frequent clinical complication was *Tinea cruris,* which is observed in 38% of participants followed by *T. capitis, T. unguium, T. mannum, T. barbae, T. faciei, T. corporis,* and *T. pedis.* Ansari et al. evaluated 316 clinical isolates of dermatophytes in Iran and reported that *T. corporis*

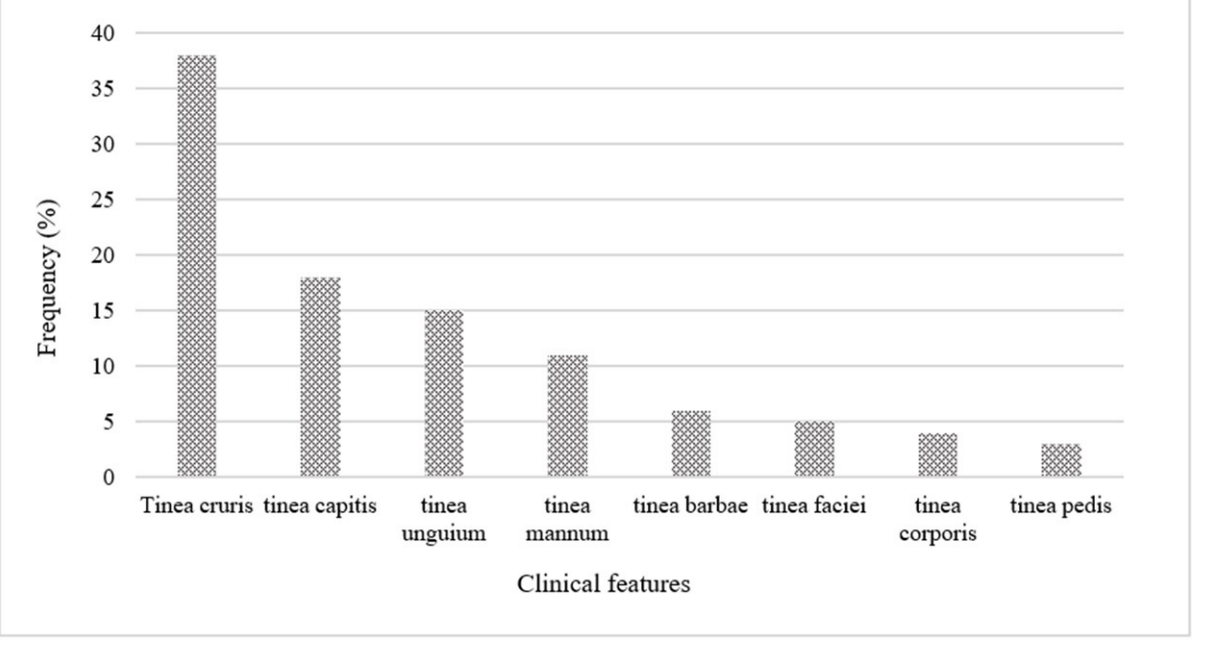

Fig. 1. Clinical features of patients in this study (percent)

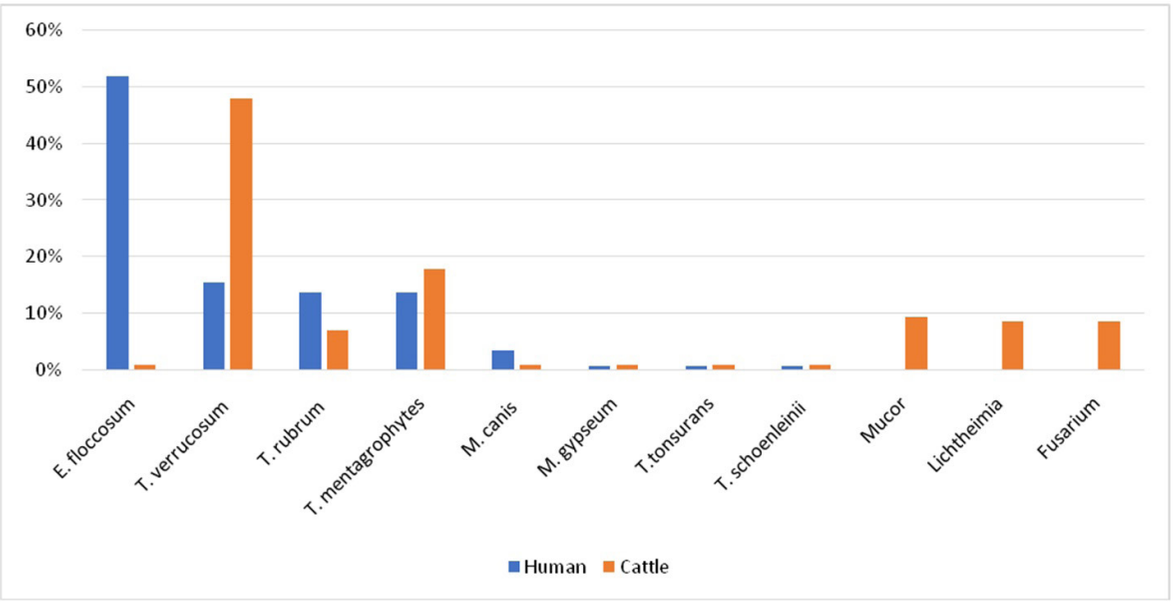

Fig. 2. Frequency of identified causative agents of dermatophytes in patients and animals.

Table 3. MIC determination of antifungal drugs against human isolated and two reference (*T. rubrum* PTCC 5143 and *T. mentagrophytes PTCC* 5054) dermatophyte strains

Dermatophyte	Antifungal agents	MIC range µg/ml	MIC_{50} µg/ml	MIC_{90} µg/ml
T. mentagrophytes (n=13)	Terbinafine	0.003-0.25	0.063 ± 0.001	0.125
	Griseofulvin	4-128	32 ± 11	64 ± 8
	Itraconazole	0.01-16	0.03 ± 0.002	12 ± 3
	Luliconazole	0.03-0.25	0.03 ± 0.001	0.25 ± 0.04
	Lanoconazole	0.01-0.25	0.03 ± 0.008	0.25 ± 0.01
	Ketoconazole	0.03-0.5	0.25 ± 0.001	0.5 ± 0.01
	Econazole	0.5-8	1 ± 0.07	4 ± 0.06
	Butenafine	0.03-4	0.12	0.17 ± 0.03
	Terbinafine	0.004-0.25	0.02 ± 0.006	0.125 ± 0.08
T. rubrum (n=13)	Griseofulvin	8-64	16 ± 3	64 ± 8
	Itraconazole	8-128	32 ± 10	64 ± 8
	luliconazole	0.5-8	1 ± 0.02	4 ± 0.17
	Lanoconazole	0.01-0.25	0.03 ± 0.003	0.25 ± 0.04
	Ketoconazole	0.03-0.5	0.25 ± 0.06	0.5 ± 0.05
	Econazole	0.01-8	0.03	0.05 ± 0.008
	Butenafine	4-128	32 ± 5	64 ± 13

The MIC_{50} (minimal concentration that inhibits 50% of isolates (Mean ± Standard Error of the Mean (SEM))) and MIC_{90} (minimal concentration that inhibits 90% of isolates) values were calculated for species

was the most prevalent type of clinical manifestation (35.2%), followed by *T. cruris* (17%) and *T. capitis* (12.8 %) (14). In another study, Didehdar et al. reported that *T. pedis* was the most common clinical form (32.1%) of clinically important dermatophytes in the north of Iran, followed by *T. cruris* (24.4%) (15). Our findings are in line with those of these investigations, and minor discrepancies should be attributed to the difference in the study population. Cattle are among the most important livestock that suffer from a va-

Table 4. MIC determination of antifungal drugs against animal isolated and two reference (*T. rubrum* PTCC 5143 and *T. mentagrophytes PTCC* 5054) dermatophyte strains

Dermatophyte	Antifungal agents	MIC range µg/ml	MIC_{50} µg/ml	MIC_{90} µg/ml
T. mentagrophytes	Terbinafine	0.003-0.25	0.08	0.11
(n = 22)	Griseofulvin	4-128	16 ± 0.5	64 ± 0.8
	Itraconazole	0.03-32	0.03 ± 0.002	12 ± 0.94
	Luliconazole	0.03-0.5	0.25 ± 0.003	0.5 ± 0.02
	Lanoconazole	0.01-0.20	0.03 ± 0.005	0.25 ± 0.02
	Ketoconazole	0.03-0.25	0.03 ± 0.001	0.25 ± 0.03
	Econazole	0.03-8	0.14	0.17 ± 0.03
	Butenafine	0.5-16	1 ± 0.07	4 ± 0.06
	Terbinafine	0.003-0.25	0. 4 ± 0.006	0.125 ± 0.06
T. rubrum	Griseofulvin	4-128	16 ± 2	64 ± 7
(n = 10)	Itraconazole	0.03-0.5	0.25 ± 0.05	0.5 ± 0.01
	luliconazole	0.5-16	1 ± 0.01	4 ± 0.12
	Lanoconazole	0.01-0.25	0.03 ± 0.001	0.25 ± 0.06
	Ketoconazole	8-128	32 ± 10	64 ± 8
	Econazole	4-128	32 ± 2	64 ± 15
	Butenafine	0.01-8	0.03	0.05 ± 0.008

The MIC_{50} (minimal concentration that inhibits 50% of isolates (Mean ± Standard Error of the Mean (SEM))) and MIC_{90} (minimal concentration that inhibits 90% of isolates) values were calculated for species

Table 5. The results of the presence of the virulence genes in *T. rubrum* and *T. mentagrophytes* strains

Genes	*T. rubrum*	*T. mentagrophytes*
Mep1	5	16
Mep2	5	16
Mep3	0	11
Mep4	22	16
Mep5	4	12
Erg11	24	21
Erg24	24	19
Erg26	0	1
ScpA	6	14
ScpB	0	0
NPII	19	18

riety of skin diseases. Previous studies showed that dermatophytosis was the most frequently contagious encountered fungal disease in cattle. The current study evaluated a total of 500 beef cattle from two sites in Tehran province, 120 collected samples. The frequency of dermatophytosis was 24% in the evaluated cattle, which was higher in summer than in other seasons ($p<0.05$, Chi-square 3.8152). The highest rate of infection was predictably found in summer compared with spring ($p<0.001$), autumn ($p<0.001$), and winter ($p<0.05$). Following our findings, Dalis et al. (16) and Guo et al. (17) reported a higher rate of infection prevalence in summer than in spring and autumn. Sebum secretion increases in summer because of higher temperature and accelerated metabolism that can change the skin surface environment and increase the chances of dermatophyte growth (17).

Lesions were found on the head (30%), face (28%), and neck (20%). A statistically significant difference was found in the prevalence of the disease between calf (12.74%, 33/259) and adult cattle (6.22%, 15/241) ($p<0.05$). The incidence of dermatophytosis among humans and livestock varies from country to country depending on many factors including climate and economy. Gue et al. reported that the lesions were more prevalent on the head (38.71%) followed by face and neck (both 20.43%), corresponding to our results. All *Trichophyton* isolates showed the same morphological properties. These findings are in accordance with that of Pal et al. (18). In addition, *T. verrucosum* was the most frequent (47.5%) isolated dermatophyte in surveys by Dalis et al. and Gue et al. (10, 17). These results are in line with our findings

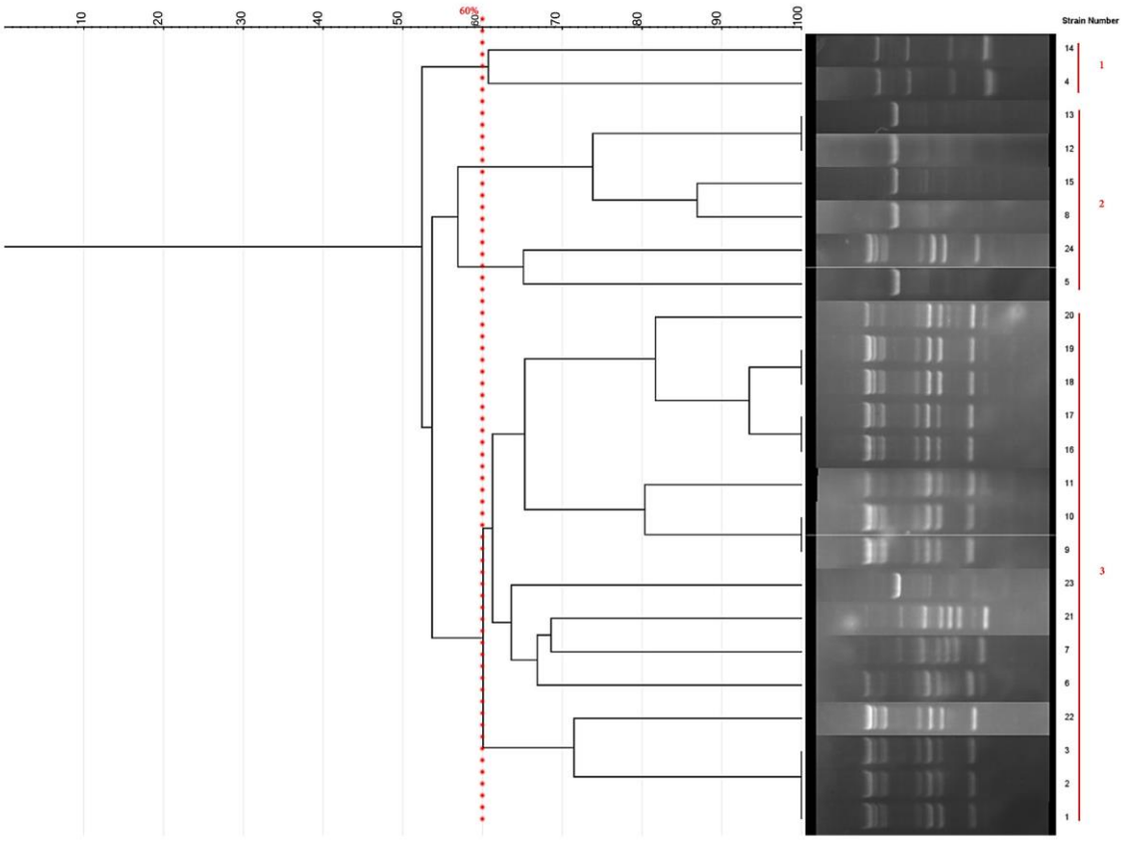

Fig. 3. Representative results of characterization of *T. mentagrophytes* strains using RAPD PCR (cut-off number: 60%). *T. mentagrophytes* isolates are divided into three genotypic groups.

indicating that *T. verrucosum* is the main pathogenic microorganism causing cattle dermatophytosis. Our results indicate that *T. verrucosum* as a zoophilic dermatophyte is endemic to this region, and its role must be further illuminated in the etiology of these infections. In addition to the global importance of dermatophytosis as the commonest superficial infection in human beings, antifungal drug susceptibility testing plays a pivotal role to determine emerging resistance profiles among etiologic trichophytons. Among those isolates without TER resistance, the MIC for terbinafine ranged from 0.003 to 0.25 μg/mL. In the present study, five *T. mentagrophytes* (15.1%) and three *T. rubrum* (14.2%) strains showed resistance to terbinafine (4-≥32 μg/mL) but remained susceptible to other agents, which is in accordance with previous reports and can be attributed to amino acid substitution in the squalene epoxidase (SQLE) enzyme (19). This level of resistance to terbinafine among investigated isolates confirms that this agent encompasses acceptable antifungal activity against various dermatophytes. This must be pointed out that the terbinafine was the most potent antifungal drug against all dermatophytes isolated from the Iranian population in previous studies (14, 19, 20). However, this significant increase in the frequency of TER-resistant species is worrisome warranting antifungal susceptibility testing and molecular examination for monitoring this emerging resistance. In line with our findings, Taghipour et al. have recently reported emerging TER resistance among trichophyton species in Iran (21). In addition, Singh et al. (22) and Hiruma et al. (23) reported high terbinafine resistance to trichophyton strains isolated in India and Japan harboring SQLE single amino acid substitutions. In our study, there was no relationship between resistance to TER and the frequency of the target genes. This indicates that the resistance must be associated with polymorphisms in the SQLE gene, as reported previously.

Our results showed that the MIC of GRI against both dermatophytes was in the range of 4-128 μg/mL. *T. mentagrophytes* showed lower susceptibility than *T. rubrum* (MIC_{50}=16 μg/mL). These findings are in line with studies of Salehi et al. (19) and

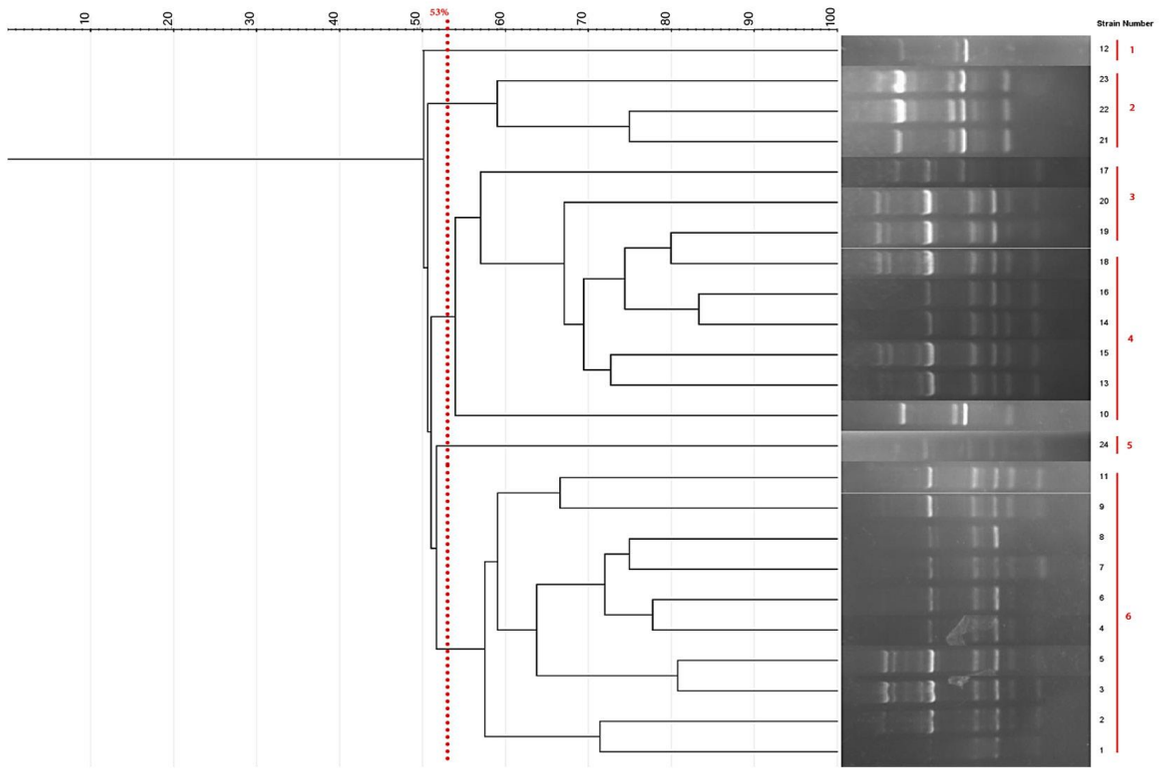

Fig. 4. Representative results of characterization of *T. rubrum* strains using RAPD PCR (cut-off number: 53%). *T. rubrumisolates* were divided into six genotypic groups.

Nowruzi et al. (20) on the Iranian population. Interestingly, the MIC_{50} for luliconazole was 0.0256 µg/mL against isolates that were resistant to terbinafine agents while this value was 0.009 µg/mL against multi-drug-resistant isolates. Baghi et al. Wiederhold et al. and Salehi et al. reported similar results about luliconazole (19, 24, 25).

Conventionally, dermatophytes such as trichophyton species were identified by direct investigation of the colony characteristics and morphological features of hyphae and spores on microscopy. This process was not accurate and feasible for various reasons. Numerous genome-based techniques have been introduced to solve these problems, and the evaluated identification techniques include the RAPD analysis, restriction fragment length polymorphisms (RFLP), Multiplex PCR, and base-sequencing. Previously, Lemsaddek et al. examined the presence of genes (*Mep1-5*) related to fungalysin and other proteases of the Subtilisin subset (*Sub1-7*) using PCR and mentioned that gene screening was necessary to investigate the infection process (26). In the present study, *Mep4* genes had the highest presence in samples compared to *Mep1, 2, 3,* and *5* in *T. rubrum,* which are related to samples isolated from the patient's groin. However, the presence of *Mep1, 2,* and *4* was the same for *T. mentagrophytes.* This necessitates the study of fungalysin virulence genes to control and reduce the damage caused by dermatophyte infections. Our results on the presence of *Mep1-5* genes in *T. rubrum* and *T. mentagrophytes* revealed that the greater frequency was related to the *Mep4* gene in *T. rubrum.*

In another study, Leng et al. stated that the proteolytic activity of *Mep4* and *Mep5* significantly decreased in comparison with wild-type and different strains, while *Mep3* was like the wild-type strain and was more active than that observed with *Mep1* and *Mep2.* These results showed that the *Mep* gene could affect the proteolytic activity of keratin. Differences in protease secretion levels, which play an important role in keratinase secretion, affect the digestive function of metalloproteases over the years (27). These results indicate the importance of further investigation of pathogenic genes in a larger and more diverse statistical population.

The study of the relationship between the frequency of pathogenic genes and resistance to antifungal

drugs showed that strains with polymorphic ERG genes were resistant to drugs, while strains without this gene family were sensitive to all antifungals ($p<0.01$). This association can be attributed to the mechanism of the polymorphic ERG gene in counteracting antifungal drugs (28). Ergosterol is a major component of yeast cell membrane sterols and is also responsible for maintaining cell function and integrity. The primary mechanism of action of some antifungal drugs, including azoles and polyanes, is to inhibit fungal cell growth by disrupting normal sterol biosynthesis, leading to a decrease in ergosterol biosynthesis (29). One of the most important genes involved in ergosterol biosynthesis is the ERG gene, and mutations in ergosterol biosynthesis genes cause the fungus to become resistant to some antifungal drugs (8). On the other hand, no statistically significant relationship was observed between the prevalence of other pathogenic genes and the pattern of resistance to antifungal drugs.

Zhong et al. studied 30 isolates of *T. rubrum* by the RAPD PCR method and found 22 indistinguishable isolates and eight isolates showed very minor differences, while Liu et al. described no differences between eight strains of *T. rubrum* using AP-PCR. In line with Khosravi et al. and Muhammed et al. (30, 31), the duplicate RAPD profiles showed 100% reproducibility in our study. Baeza et al. (12) analyzed the genetic properties of dermatophytes and concluded that RAPD PCR was a proper method that could be used in epidemiological studies.

CONCLUSION

Taken together, the results of the study indicate the remarkable rate of resistance to TER in isolated dermatophytes that requires further investigation. The study of genome polymorphism showed that virulence genes and ITS region were valuable genetic indicators in the evaluation of the genome structure of dermatophytes, which can improve our understanding of the epidemiology of these fungi to provide more effective prevention policies.

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