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Molecular identification of *Trichophyton mentagrophytes* isolated from tinea corporis in Kirkuk City, Iraq

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ABSTRACT

This study used to be conducted in the Graduate Studies Laboratory at the College of Science, Kirkuk University in Iraq from 11/7/2021 to 25/5/2022 with the aim of investigating the fungi causing motive pores and skin ailments, i.e., dermatomycosis in the human physique (tinea corporis) and the incidence of *Trichophyton mentagrophytes*. A total of 120 samples were collected from sufferers referred to the Dermatology Consultant at Azadi Teaching Hospital and some non-public clinics in Kirkuk, age groups ranging from 1 to 60 years and both genders. All samples were examined and recognized using traditional methods and using culture media for the fungi. The direct microscopic examination of the fungi showed positive results, with an infection rate of 66.66%, while the laboratory culture showed positive results, with 49.16%. The results of the phenotypic examinations of the isolated dermatophytes showed that they belong to the two genera, *Trichophyton* and *Microsporum*. The results showed that *Trichophyton* was the most diagnosed specimen compared to *Microsporum*. The least patient specimen was diagnosed, and 5 species of *Trichophyton* were isolated, *T. mentagrophytes* exhibited the highest prevalence (9.2%), while the lowest belonged to *T. interdigitale* (2.5%). In the case of *Microsporum*, two species were isolated, so that, the infection rate of males (33.33%) was higher than that of females (15.83%). The result showed the highest infection rate of *T. mentagrophytes* in the age group 21-30 years, while the lowest among the group 50-61. After final identification of *T. mentagrophytes* by PCR, the consequences of the PCR assay were sent to the Genebank website to verify the kind of fungus through comparing it with the fungi registered at this site.

Keywords: Dermatophytes, *Trichophyton mentagrophytes*, PCR.
Article type: Research Article.

INTRODUCTION

Dermatophytes are a team of fungi that have the capability to invade the stratum corneum of the epidermis, keratinized tissues such as pores and skin and nails, human hair, wool, feathers, and horns in animals (Begum *et al*. 2020). However, dermatophytosis infection is generally confined to the dead keratinized layers of the skin due to host defence responses to the invasion of fungi in immunocompetent individuals and as a result of the ability of these fungi to secrete the keratinase enzyme that has the ability to break down and dissolve the keratin layer present in the skin, hair and nails (Jochen & Yvonne 2005). This has given great importance to these fungi from a medical point of view, since the infection with dermatophytosis is in the form of circular spots or inflammatory patches with high and distinct edges, as well as the appearance of scaling, redness and itching in the affected area of the skin along with hair loss (Ellis 1994). This may happen since dermatophytes pick out secure areas for their growth, such as superficial keratinocytes, due to escaping from the host's defence mechanisms rather than the inability to invade deeper tissues. Therefore, dermatophytes present in people with weakened immunity, such as extreme diabetes, may exhibit additionally growth to deep and subcutaneous infections. The classification of cutaneous vertebrates into three genera, i.e., *Microsporum*, *Epidermophyton* and *Trichophyton*, dominates in skin infections in humans and animals. *Trichophyton* is one of the most common dermatophytes genera (Tyagi 2016). About 30 species act as causative agents of dermatophytosis, e.g., *T. mentagrophytes*, which constitute the

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majority of isolates of the fungi, followed by *T. rubrum*, *T. tonsurans*, *M. canis*, *M. gypseum* and *E. floccosum* (Das *et al.* 2007).

MATERIALS AND METHODS

Sample collection

A total of 120 medical samples were gathered from people infected with dermatophytosis from the Consultant Dermatologist in Kirkuk City, Iraq for the period between 7/11/2021 and 25/5/2022, forwarded from the specialist physicians, for the age groups ranging from 1 to 60 years and for both genders. Skin samples were taken by scraping, where the affected area was sterilized with 70% ethyl alcohol, and then the scales were scraped from the edge of the fungus-infected focus using a sharp sterile blade, followed by transferring to the Fungi Laboratory, College of Science, University of Kirkuk for examination and cultivation, and allocating a questionnaire to every affected person containing some scientific and personal data about the auditors. Diagnosis of isolated samples was performed by direct method and culture of samples according to (Ellis (1994).

Molecular diagnostics *of T. mentagrophytes*

DNA extraction and specialized replication by polymerase chain reaction (PCR) were carried out from colonies of *T. mentagrophyte*s.

Genomic DNA extraction protocols

The DNA extracted from a single pure colony of *T. mentagrophytes* and actively growing was employed using the Quick-DNATM Fungal/Bacterial Miniprep Kit Protocol, provided by Zymo Research in the United States of America, and the extraction was carried out in accordance with the company's guidelines as follows:

PCR amplification protocols

Single nucleotide polymorphisms genotyping

The utility of PCR approach based totally on the difference in the ITS region was once carried out to verify the prognosis of fungal isolates of *T. mentagrophytes*. The fungal DNA was used to be extracted and the ITS target region used to be amplified using each of the primers (Table 1).

Table 1. Sequence of nitrogenous bases in the primers used in the DNA amplification process isolated from dermatophytes according to (Abbas *et al*. 2020).

Primer	Sequence	Tm	GC	Product size
Forward	5′- TCCGTAGGTGAACCTGCGG -3′(	60.3 ℃	50 %	550 base pair
Reverse	5′ TCCTCCGCTTATTGATATGC-3′)(	57.8 ℃	41%	550 base pair

Preparation of the reaction solution for PCR: A PCR response combination (25 µL) was prepared (Table 2).
Then the aspects of the PCR reaction mixture depicted in Table 2 were positioned in extraordinary 0.2-mL tubes containing the relaxation of the PCR response components. All tubes were transferred to the Vortex mixing device for two min, and then positioned in the Thermocycler PCR according to the perfect stipulations for thermal cycles.

RESULTS AND DISCUSSION

A total of 120 patient samples were collected from clinically-diagnosed infections by specialized physicians for patients with dermatophytes and some private clinics from 7/11/2021 to 25/5/2022 for age groups between 1 and 60 years and both genders. The results of direct microscopic examination appeared. A total of 80 positive skin samples were found with a prevalence of 66.66%, and 40 negative (33.3%), while the results of laboratory culture on the medium of SDA showed the presence of 59 positive- and 61 negative- samples (49.16% and 50.84%, respectively). In Table 4, the appearance of negative samples in microscopic examination may be due to an error in the sampling process or the method of storing the sample during its transportation to the laboratory, or due to

taking a small amount of the sample. So that, it might be insufficient to exhibit a positive result, in addition to the presence of saprophytic fungi. Saprophytic fungi may grow with dermatophytes at the site of infection and may compete with them for nutrients and prevent dermatophytes from growing on the SDA medium (Habeb 2016).

Table 2. Volumes of PCR (polymerase chain reaction).

Component	Volume
(Taq PCR PreMix)	5 µL,,
(DNA)	(1.5 µL)
(Forward primer)	10 picomols μL^{-1} (1 µL)
(Reverse primer)	10 picomols μL^{-1} (1 µL)
(Distill water)	(16.5 µL)
(Total volume)	(25 µL)

Table 4. Represents the infection of dermatophytes based on direct microscopic examination and laboratory culture.

Total summation	Negative Samples		Positive Samples		Test type
100%	33.3%	40	66.7%	80	Direct microscopy
100%	50.84%	61	49.16%	59	Laboratory culture

Prevalence of dermatophytes according to genders and age groups

According to the results of the current study, those infected with dermatophytes in the age group 30-21 years exhibited the highest prevalence of 49.2%, and in this age group, the number of infected males was higher than females (32.5% vs. 16.7% respectively). It is in agreement with Parameswari (2015), who found the highest prevalence in the age category of 30-21years by 40% and also in agreement with Ahmed (2022). The elevation in the prevalence of dermatophytes in this age group may be due to an upraise in the number of infected males because of heavy sweating, frequent exposure to hard work extending to long hours, not changing clothes and not washing for a relatively long period, and their frequent exposure to heat and humidity, especially those in prisons (Todaro *et al*. 1983; Table 5). This study does not in agreement with Mohammed (2019), how found that the age groups between 6 months and 9 years exhibited the highest prevalence (32.6%). Moreover, it was not in agreement with Nussipov *et al*. (2017), who reported that the elevation in the prevalence of children may be due to the lack of saturated fatty acids in their bodies, which affects the protection against fungal diseases and thus leads to the occurrence of skin injuries in children, compared to adults. The least prevalence (3.3%) was observed in the age group of 60-51 years. It is consistent with the results of Al-Zubaidi (2019), who reported the lowest prevalence (0.32%) in the age group over 50 years, but not with those of Yehia (1980).

Types of isolated dermatophytes during the study

The isolated dermatophytes were diagnosed based on the cultivar characteristics of the developing colonies, such as the colour, nature of the farm, size, the back side of the plate, and microscopic characteristics such as conidia shape, size and fungal hyphae, where 40 isolates (33.33%) belonging to dermatophytes were diagnosed, of those, 4 isolates were for females (3.33%) and 36 isolates (30%) for males, and 7 species belonged to two genera: *Microsporium* and *Trichophyton*. The latter was more common than the former (25.83% vs. 7.5% respectively; Table 6). The fungus *T. mentagrophyton* was more common with the prevalence of 9.2% (11 isolates), followed by *T. tonsorans* with 5.83% (7 isolates), *T. rubrum* with 5% (6 isolates), *M. canis* with 4.2% (5 isolates), as well as *T. verrucosum* and *M. ferrgineum* with 3.3% (4 isolates) for each. Furthermore, *T. interdigitale* exhibited the lowest prevalence (2.5%; 3 isolates; Table 7). Studies indicate that the dominance of the genus *Trichophyton* over *Microsporum* is that the former includes many species, some of them are anthropophilic, zoophilic and geophilic (Kannan *et al*. 2006). The genus *Trichophyton* is one of the most vital and most frequent genera (Fig.

1). Monod *et al*. (2002) reported that among the ten species pathogenic to humans and isolated in Europe, the fungi *T. mentagrophytes* and *T. rubrum* can be observed in common, and it was found that the genus Trichophyton is responsible for 75% of cutaneous fungal infections (Norris *et al*. 1999).

Table 5. Prevalence of different age groups infected with dermatophytes.

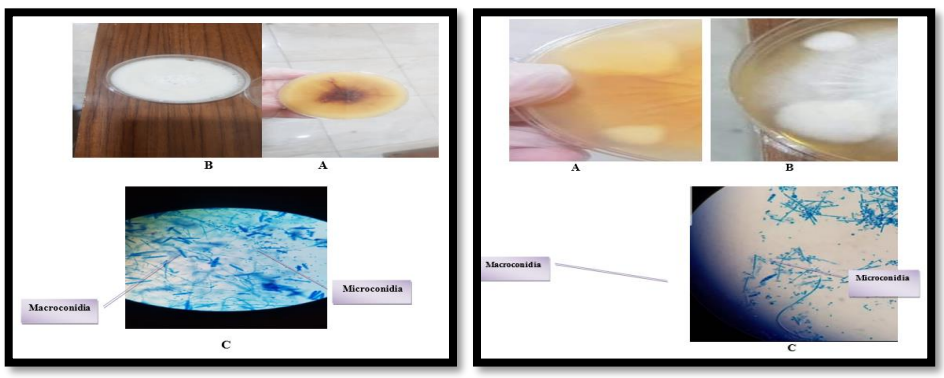
Prevalence (%)			Total	Sex		Age group
Total	Female	Male		Female	Male	(year)
6.7	2.5	4.2	8	3	5	10-1
12.5	4.2	8.3	15	5	10	20-11
49.2	16.7	32.5	59	20	39	30-21
20	6.6	13.3	24	8	16	40-31
8.3	2.5	5.8	10	3	7	50-41
3.3	0.8	2.5	4	1	3	60-51
100	33.3	66.7	120	40	80	Total

Table 6. The genera of dermatophytes.

Prevalence (%)	Number	Genus of dermatophytes
25.83	31	*Trichophyton* spp.
7.5	9	*Microsporum* spp.
33.33	40	Total

Table 7. Dermatophytes species isolated from patients

Prevalence (%)	Number	Dermatophytes species
%9.2	11	*T. mentagrophytes*
%5.83	7	*T. tonsorans*
%5	6	*T. rubrum*
%4.2	5	*M. canis*
%3.3	4	*T. verrucosum*
%3.3	4	*M. ferrgineum*
%2.5	3	*T. interdigitale*
%33.33	40	The total



A **B**

Fig. 1. (A) *T. mentagrophytes* growth on SDA medium; (B) *M. canis* growth on SDA medium.

Molecular diagnosis of *T. mentagrophytes*

PCR (polymerase chain reaction)

The isolated dermatophytes species were identified with the aid of relying on phenotypic, microscopic and laboratory characteristics, and the validity of the diagnosis of *T. mentagrophytes* verified using the ITS region. It is the most broadly sequenced region in the molecular surroundings of fungi, and has been recommended as a universal fungal sequence. It has usually been more useful for molecular study at the level of species to genus and even within species (Fujita *et al*. 2001; Al-Enezi & Jamil 2023; EL-Saman *et al.* 2023), and employing each of the primers, ITS1 and ITS4 to diagnose *T. mentagrophytes* using a technique. The PCR in the present study was used to diagnose 6 isolates of moulds relying on the initiator (Fig. 2). The traditional adopted for diagnosing moulds and species of the genus *Trichophyton* which is based on determining phenotypic criteria, is insufficient due to the overlap of these criteria with other species that are categorized within different species of the equal genus *Trichophyton*, as properly as the genetic version amongst them. To a sure group, it differs in its phenotypic traits and boom characteristics. The difference does now not always imply a distinction in the genetic structure, specifically between farms and isolates that belong to the equal sex and species. Rather, it can also be the end result of the response of farms and isolates to environmental circumstance (Mohieddin & Jigan, 2013).

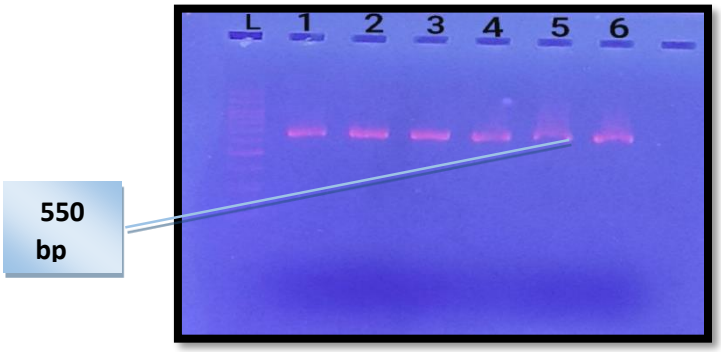


Fig. 2. The result of the PCR technique for *T. mentagrophytes* (6 = PCR Sample-L = Ladder, 1) where L: 550 bp.

Dermatophyte DNA extraction

There are many ways to extract DNA from eukaryotic cells. All of these methods were performed at low temperatures and with sterile tools and solutions to ensure contamination-free extraction (Mahmoudabadi *et al*. 2013). The results of DNA amplification were obtained by PCR technique on *T. mentagrophytes* using ready-made kit from Quick-DNATM Fungal/Bacterial Miniprep Kit Protocol, then electrophoresis in agarose gel, and detection using Red safe nucleic DNA dye acid staining and examination under UV rays. All isolates of *T. mentagrophytes* contained a single bundle of extracted DNA by taking the same location on the agarose plate. The quantity and purity of the extracted DNA was measured using a Nanodrop device, where all the isolates appeared with a single molecular size of 550-bp pair base.

Diagnosis of the isolates below find out about based totally on the sequence of nitrogenous bases of the ITS

Vicinity A prognosis used to be made for six DNA samples extracted from *Trichophyton* farms by figuring out and examining the outcomes of the nitrogen base sequences analysis in the NCBL BLAST application (National Centre for Biotechnology Basic Local Alignment Search Tool) to find out their similarity with the outcomes in the global gene bank after obtaining the sequence. The nucleotides of the DNA bundle of local isolates and their comparison with the sequences of the same ITS location, as well as neighbourhood *Trichophyton* isolates were registered through NCBI BLAS (Table 8). The document recording the nitrogenous bases for all isolates of *Trichophyton* in the National Centre for Information and Biotechnology (NCBI) showed the classification of fungus as follows: Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Eurotiomycetes; Eurotiomycetidae; Onygenales; Arthrodermataceae; *Trichophyton*. Where *T. mentagrophytes* showed congruence with the results in the gene bank, and the effects of analysing the nitrogen bases and comparing it with the isolates registered in NCBL proved that all fungal isolates isolated from contaminated sufferers are isolates belonging to *T. mentagrophytes*, and this end result confirms the phenotypic and microscopic diagnosis. These received outcomes are in settlement with the results acquired by Maikhan *et al*. (2018). The isolates underneath

learn about were registered at the NCBI Information and Biotechnology Centre, according to the identification numbers for every isolate, which files the results obtained (Table 9).

Table 8. Of the international isolates and their accession numbers in NCBI, which were in contrast with them thru BLAST website, showing the locations of heterogeneity and their location, in addition to the percentage of congruence with the isolates under find out about.

Trichophyton mentagrophytes

No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1				ID: ON024352.1	*Trichophyton mentagrophytes* isolate DERM RML5 internal transcribed spacer 1	100%
2				ID: ON024352.1	*Trichophyton mentagrophytes* isolate DERM RML5 internal transcribed spacer 1	100%
3				ID: ON024352.1	*Trichophyton mentagrophytes* isolate DERM RML5 internal transcribed spacer 1	100%
4	Transversion	375	(C,G)S/C	ID: ON024352.1	*Trichophyton mentagrophytes* isolate DERM RML5 internal transcribed spacer 1	99%
5				ID: ON024352.1	*Trichophyton mentagrophytes* isolate DERM RML5 internal transcribed spacer 1	100%
6				ID: ON024352.1	*Trichophyton mentagrophytes* isolate DERM RML5 internal transcribed spacer 1	100%

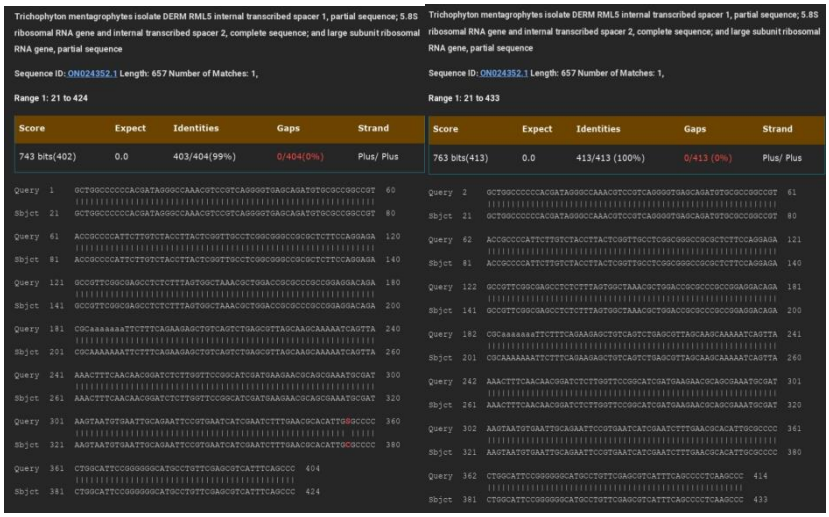
Table 9. registration numbers of isolates underneath learn about at the NCBI Information and Biotechnology Centre.

Sequence ID	Accession	Time
j [1]	OP721054	Oct. 31, 2022
j [2]	OP721055	Oct 31, 2022
j [3]	OP721056	Oct 31, 2022
j [4]	0P721057	Oct 31, 2022
j [5]	OP721058	Oct 31, 2022
j [6]	OP721059	Oct 31, 2022

Determination of the nitrogenous base sequence of the products of a specialized replication chain reaction

The nitrogenous base sequences of the genomic DNA samples of the six isolates from yeasts different than the base sequences of the fashionable traces recorded in the Diagnosed Gen Bank were decided and in contrast. For the National Centre for Biotechnology Information NCBI using the DNA BLAST program, the isolates were given codes isolation j [1], j [2], j [3], j[4], j [5], j[6]. The result of the diagnosis by PCR technique showed that these isolates (j[1], j[2], j[3], j[5], j[6]) belong to the fungus *T. mentagrophytes*, since there was convergence during the analysis process of these sequences with the sequences recorded in the Gene bank. The result of the analysis using the DNA BLAST program showed a 100% match between the sequences of these isolates and the sequences of nitrogenous

bases of the standard fungal strain (Fig. 3A). However the result of j4 confirmed that matching 99% between these sequences and the sequences of the fungal widespread stress registered in the gene financial institution (Fig. 3B).



A **B**

Fig. 3. (A) Comparison of the sequences of nitrogenous bases between the regional isolates (j 1, j 2, j 3, j4, j 5, j6) and the customary pressure recorded in the Gen bank; (B) Comparison of the sequences of nitrogenous bases between the close by isolate j4 and the favoured strain recorded in the Gen bank.

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