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Morphological and Molecular Identification of Dermatophytes Isolated from Clinical Samples Using ITS rDNA Sequencing

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Abstract

Background: Dermatophytes are the principal causative agents of superficial fungal infections and remain an important public health concern. Accurate species identification is essential for epidemiological characterization. **Aim:** The present study aimed to isolate and identify dermatophytes recovered from patients with suspected dermatophytosis using conventional morphological and molecular techniques. **Methodology:** A total of 80 clinical specimens were collected and cultured on Sabouraud Dextrose Agar (SDA). The recovered isolates were identified based on their macroscopic and microscopic characteristics, followed by molecular confirmation through amplification and sequencing of the internal transcribed spacer (ITS) region. **Results:** Phylogenetic analysis was performed to determine the genetic relationships among the representative isolates and reference strains. Seventy fungal isolates (87.5%) were recovered, of which dermatophytes accounted for 92.9%. *Trichophyton indotineae* was the predominant species (57.1%), followed by *Microsporum canis* (35.7%), while *Chaetomium globosum* represented 7.1% of the isolates. The morphological characteristics of *T. indotineae* and *M. canis* were consistent with their classical taxonomic descriptions. Molecular analysis confirmed species identification, with ITS sequences showing more than 99% similarity to reference strains deposited in GenBank. **Conclusions:** Phylogenetic analysis demonstrated that the representative Iraqi isolates clustered closely with international strains, indicating limited genetic

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diversity. These findings demonstrate that the integration of conventional morphological examination with ITS-based molecular identification provides a reliable approach for the accurate identification and epidemiological characterization of dermatophytes.

Keywords: Dermatophytes; *Trichophyton indotineae*; *Microsporum canis*; ITS rDNA sequencing.

Introduction

Dermatophytes are a group of keratinophilic filamentous fungi that have the ability to colonize keratinized tissues, including the skin, hair, and nails of both humans and animals. They degrade and utilize keratin as a nutrient source, leading to the development of fungal skin infections with diverse clinical manifestations and notable public health implications [1]. These fungi primarily include the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*, which represent the most common causative agents of dermatophytosis. Among *Trichophyton species*, *T. mentagrophytes*, *T. interdigitale*, and *T. rubrum* are frequently reported as major human pathogens. In contrast, *Microsporum canis* and other zoophilic species are known for their ability to be transmitted from animals to humans, causing zoonotic infections [2].

Traditional identification of fungi has relied primarily on morphological and microscopic characteristics, including colony morphology, pigmentation, and reproductive structures. Although these methods are widely used and cost-effective, they often lack accuracy at the species level due to phenotypic variability and morphological similarities among closely related species. In contrast, molecular identification methods have become essential tools in modern mycology [3].

The diagnosis of pathogenic dermatophytes is considered an important factor not only in determining the epidemiology of the disease, but also in achieving successful treatment (Sahgal & Magan, 2008). molecular methods have been widely used for the identification of dermatophytes, including polymerase chain reaction (PCR), real-time PCR (RT-PCR), PCR-enzyme immunoassay (PCR-EIA), nested PCR, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and random amplified polymorphic DNA PCR (RAPD-PCR) techniques [4].

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Materials and Methods

Study Setting and Sample Collection

A total of 80 clinical specimens were collected from patients suspected of dermatophytosis during the study period extending from January 5 to September 1, 2026. Samples were obtained from infected skin, hair, and nail sites at Imam Al-Hussein Medical Hospital and private dermatology clinics in Karbala, Iraq. All specimens were collected under the direct supervision of a dermatologist. The patients varied in age and sex.

Culture and Morphological Identification

Clinical specimens were cultured on Sabouraud Dextrose Agar (SDA). Recovered fungal isolates were initially identified according to their macroscopic colony characteristics and microscopic features, including hyphal structures, macroconidia, and microconidia observed by light microscopy.

Molecular Identification

For the accurate molecular identification of representative fungal isolates *of Microsporum, Trichophyton* and *Chaetomium*., genomic DNA was extracted from a 7-day-old pure culture using a standard protocol [4]. Phylogenetic analysis was done using the internal transcribed spacer (ITS) region to confirm further the identity of the fungal pathogen isolated. The ITS region was amplified with primer pair ITS1/ITS4 [6]. The PCR conditions and protocol are described previously [7]. The amplified PCR products were purified and sequenced by Macrogen, Inc. (Seoul, South Korea). All experiments and identification procedures were performed in triplicate.

Sequence and Phylogenetic Analysis

The sequences were edited and curated using BioEdit v7.0.9.0 and A Plasmid Editor (ApE) v 2.0.61. Sequence similarity searches were conducted using the BLASTn algorithm against the fungal database at NCBI. Phylogenetic analysis was conducted using MEGA v11.0.13, with multiple sequence alignments performed via MUSCLE. A neighbor-joining (NJ) phylogenetic tree was constructed with 1,000 bootstrap replicates to assess branch support. The confirmed nucleotide sequences of the fungal isolate were subsequently submitted to GenBank, where accession numbers were assigned [8].

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Results

Isolation and Identification of Fungal Isolates

Out of the 80 clinical samples examined, 70 fungal isolates were recovered, representing an isolation rate of 87.5%. Based on morphological and molecular identification, 40 isolates (57.1%) belonged to the genus *Trichophyton*, while 25 isolates (35.7%) were identified as members of the genus *Microsporum*. In addition, five isolates (7.1%) were identified as *Chaetomium globosum*, a non-dermatophyte fungus isolated from clinical specimens collected from the groin region of affected patients. Overall, dermatophytes (*Trichophyton* and *Microsporum*) accounted for 65 isolates (92.9%) of the total fungal isolates recovered.

In figure 1, the morphology and microscopic characteristics support the molecular findings. *T. indotineae* colonies on SDA appeared creamy initially, turning yellow-orange with age, with a yellow-orange reverse pigmentation. Microscopy revealed spindle-shaped, thick-walled macroconidia with terminal asymmetry and rare microconidia.

In Figure 2, the colony morphology supports the molecular findings. *M. canis* colonies on SDA appeared white with a cottony to velvety surface and a slightly raised center on the obverse (A), while the reverse (B) exhibited yellowish to light brown pigmentation. Microscopic examination revealed abundant round to *pyriform microconidia* smooth thin – walled cigar – shaped macroconidia, and occasional spiral hyphae(c), confirming the morphological characteristics of *T. indotineae.*

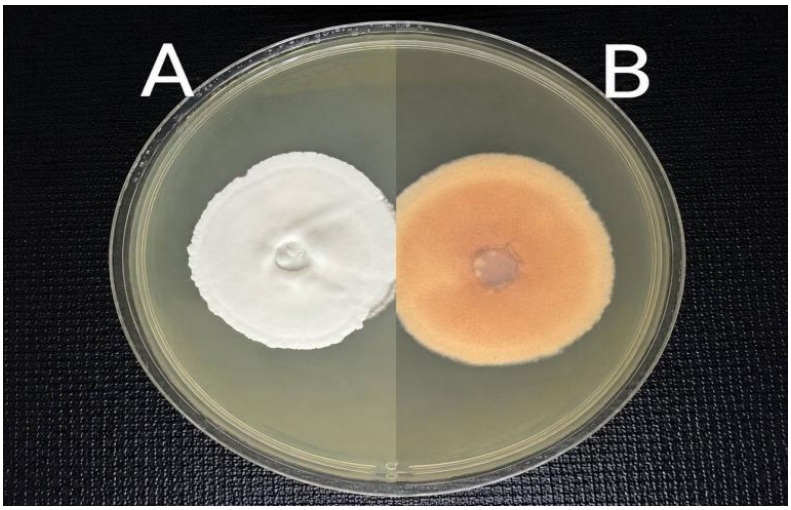

Fig. 1: Colony morphology of *T. indotineae* on SDA after 10 days at 28°C. A: Surface view – white to cream velvety texture. B: Reverse side – off-white to tan pigmentation.

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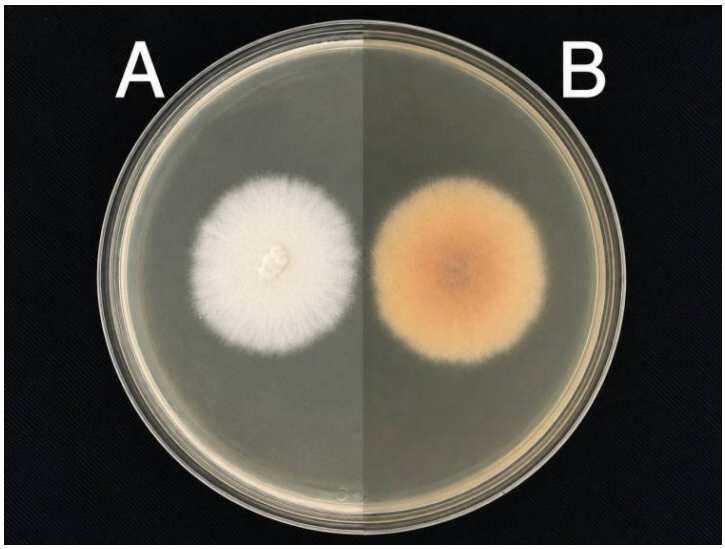

Fig .2: Colony morphology of *M. canis* on SDA after 10 days at 28°C A: Surface view – creamy to yellow-orange colonies. B: Reverse side – yellow-orange pigmentation.

Molecular Identification and Phylogenetic Analysis

The morphological identification was confirmed molecularly through amplification and sequencing of the ITS region. These representative sequences were deposited and have Genbank Accession numbers PV990886.1 for *M. canis* and PZ157928.1 for *Trichophyton indotineae*.

The BLASTn analysis presented substantial evidence of > 99% identity of these sequences with numerous corresponding sequences of international *M.canis* strains and *T. indotineae*. The phylogenetic analysis demonstrated clustering of the representative isolate, *T. indotineae* isolates Z1, Z2, identified in this study, with various *T. indotineae* depending on multiple alignments of the ITS sequence (figure 3).

Likewise, the Z3 isolates of *M. canis* were grouped with various global strains of *M. canis* (figure 4)

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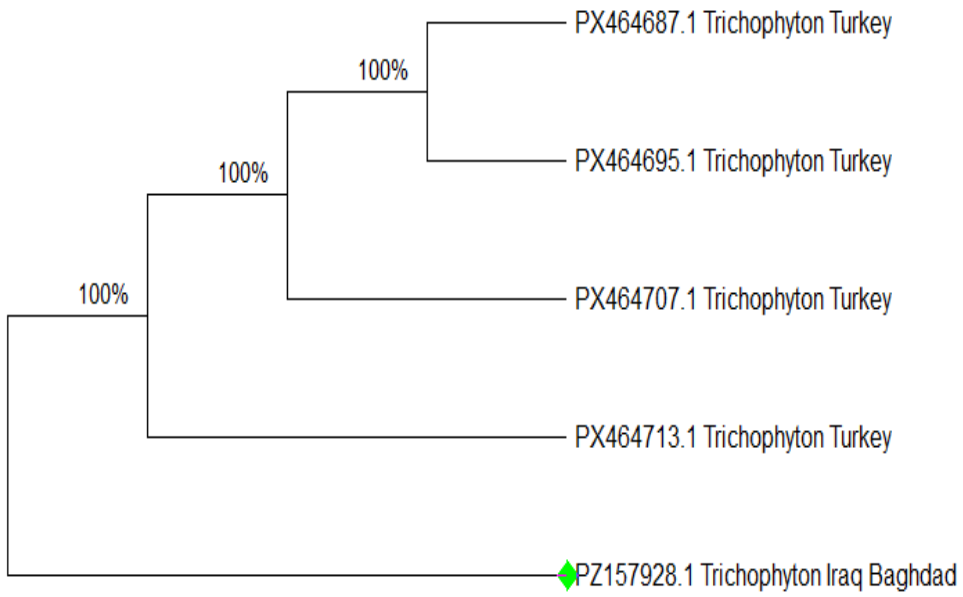

Fig.3 Phylogenetic analysis based on the ITS region sequences demonstrated that the *T.indotineae* isolates obtained in this study clustered with reference *T.indotineae* strains retrieved from GenBank, indicating a high degree of genetic relatedness. The evolutionary relationships were inferred using the Neighbor-Joining method under the Maximum Composite Likelihood model implemented in MEGA v11.0.13. Ambiguous positions were excluded using the pairwise deletion option, resulting in a final dataset of 433 nucleotide positions. These findings further confirmed the molecular identification of the isolates as *T.indotineae* .

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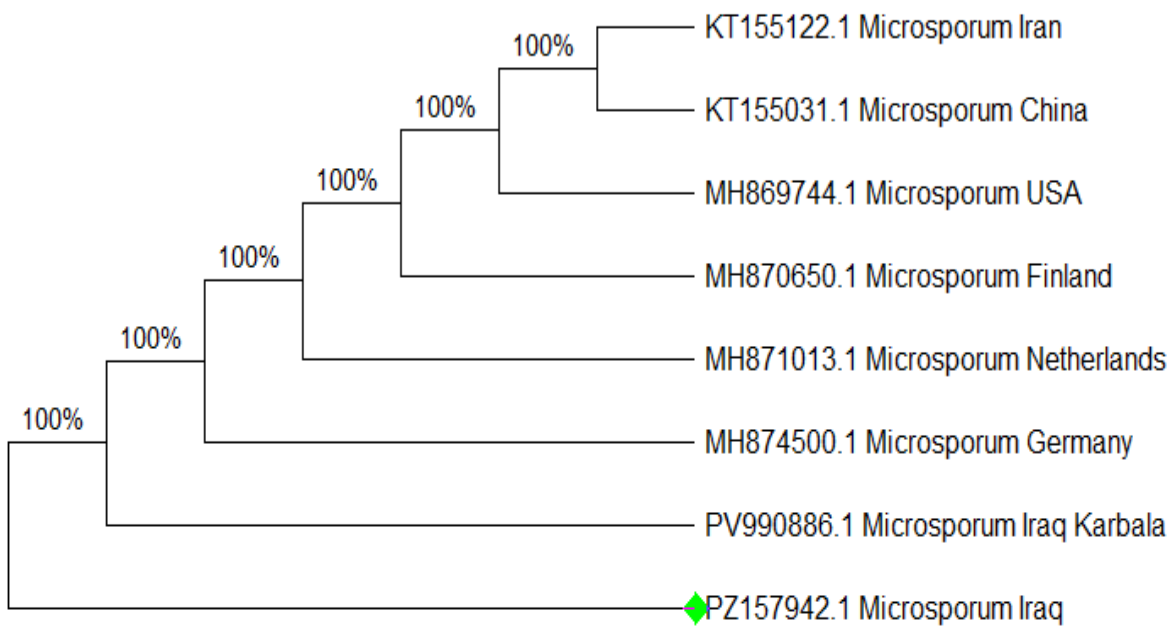

Fig.4 Phylogenetic analysis was conducted in MEGA v11.0.13 using the Maximum Composite Likelihood model. A total of eight nucleotide sequences were analyzed, including the 1st, 2nd, and 3rd codon positions together with non-coding regions. Pairwise deletion was applied to eliminate ambiguous positions, yielding a final alignment of 420 nucleotide positions. The resulting phylogenetic tree was used to infer the evolutionary relationships among the analyzed sequences.

DISCUSSION

The present study demonstrated a high fungal isolation rate (87.5%), with dermatophytes accounting for 92.9% of the recovered fungal isolates. *Trichophyton indotineae* was the predominant species (57.1%), followed by *Microsporum canis* (35.7%). The predominance of *T. indotineae* reflects its recent emergence as a major etiological agent of dermatophytosis in many countries, whereas the persistence of *M. canis* indicates the continued importance of zoonotic transmission. These findings are consistent with those reported by Uhrla and Jabet [9] who described the increasing prevalence of *T. indotineae* and the continued epidemiological significance of *M. canis*. The colony morphology and microscopic characteristics of *T. indotineae* and *M. canis* agreed with the classical taxonomic descriptions of both species, supporting the usefulness of conventional mycological methods for preliminary identification. However, molecular confirmation remains essential for differentiating closely related

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dermatophytes. Similar findings were reported by de Hoog and Schoch [10]. ITS sequencing confirmed the morphological identification, with more than 99% sequence similarity to reference strains deposited in GenBank. Moreover, phylogenetic analysis showed that the Iraqi isolates clustered closely with international strains, indicating limited genetic diversity and supporting the global distribution of these dermatophytes [9].

Conclusion

Trichophyton indotineae was the predominant dermatophyte isolated in the present study, followed by *Microsporum canis*, confirming the major role of dermatophytes in superficial fungal infections. Morphological identification, supported by ITS sequencing and phylogenetic analysis, provided accurate species identification and demonstrated close genetic relationships with international reference strains. These findings emphasize the value of integrating molecular techniques with conventional methods for reliable diagnosis and epidemiological monitoring of dermatophytes.

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