http://ijmpes.com doi:10.34172/ijmpes.6269

Open Access Original Article

APMI Press Aras Part Medical International Press

Int J Med Parasitol Epidemiol Sci 2026;7(2):111-118

eISSN 2766-6492

International Journal of Medical Parasitology & Epidemiology Sciences

Molecular Identification and Phylogenetic Analysis of *Trichophyton indotineae* Isolates from Patients with Dermatophytosis in Babylon Province, Iraq

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Abstract

Introduction: Trichophyton indotineae has emerged globally as a highly contagious, terbinafine-resistant dermatophyte causing treatment-refractory superficial mycoses, posing a significant public health threat.This study evaluated the epidemiology of dermatophytosis in Babylon Province, Iraq, and performed molecular identification and phylogenetic analysis of local clinical isolates.

Methods: Clinical specimens from 258 patients aged 1 to 71 years were analyzed. Following phenotypic characterization, genomic DNA was extracted from three representative isolates. Polymerase chain reaction amplified the internal transcribed spacer region, followed by DNA sequencing and neighbor-joining phylogenetic analysis.

Results: Tinea corporis was the most prevalent clinical presentation (37.89%), followed by Tinea pedis (16.69%) and Tinea capitis (15.09%). Infections were significantly more common in rural areas (57.4%) and individuals aged over 13 years (73.5%). Gender-specific variations were observed, with Tinea pedis dominating in males (85.6%) and Tinea corporis in females (63.9%). Molecular and phylogenetic analyses of the three isolates confirmed 100% sequence identity with global T. indotineae reference strains (GenBank ON528187.1), clustering them within the T. indotineae clade.

Conclusion: This study provides the first molecular confirmation of T. indotineae in Babylon Province, Iraq. The findings highlight the critical need for molecular surveillance and antifungal susceptibility testing to manage resistant dermatophytosis.

Keywords: Trichophyton indotineae, Dermatophytes, DNA sequencing, Phylogenetic analyses

Received: April 23, 2026, **Revised:** June 14, 2026, **Accepted:** June 22, 2026, **ePublished:** June 29, 2026

Introduction

Recently, *Trichophyton indotineae* has been described as an anthropophilic dermatophyte that has emerged as an important cause of extensive, often treatment-refractory dermatophytoses. First characterized in 2020, *T. indotineae* is a member of the *T. mentagrophytes/T. interdigitale* complex but can be distinguished by characteristic ITS sequences and other molecular markers (1-6). Its emergence has been linked to the extensive overuse of topical corticosteroids and antifungals (7), many isolates show reduced susceptibility or resistance to terbinafine, the standard systemic therapy for dermatophytosis (8-11).

Reports from Europe, North America and other regions indicate that *T. indotineae* has spread beyond South Asia, producing outbreaks and isolated cases that pose diagnostic and therapeutic challenges. The close genetic similarity among *T. mentagrophytes*, *T. interdigitale* and *T. indotineae* means that routine phenotypic methods are often insufficient; ITS sequencing and targeted molecular assays improve species-level identification and support epidemiological surveillance (4, 8).

In Iraq, dermatophytoses are common, but data on the species distribution and antifungal susceptibility of dermatophytes remain limited. Determining whether *T. indotineae* is present in local clinical isolates is important because its presence would have direct clinical implications (potential terbinafine resistance) and public health implications (the need for infection control and antifungal stewardship. Therefore, the aim was to evaluate the epidemiology of dermatophytic infections in Babylon Province, determine the distribution of infections by age, gender, and residence, and report ITS sequencing and phylogenetic placement of three clinical isolates collected in Babylon Province.

Materials and Methods

Sample Collection and Identification

A study was conducted on 258 patients (aged 1–71 years) with suspected dermatophytosis attending dermatology clinics in Babylon Province, Iraq. Clinical samples included skin scrapings, hair, and nail clippings. Informed consent was obtained and approved by the Ethics Committee of Mustansiriyah University. Samples were cultured on Potato Dextrose Agar (PDA) and Sabouraud

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Dextrose Agar (SDA) at 28°C for up to 14 days. Colony morphology and microscopic features were examined for preliminary identification.

DNA Extraction

The FavorPrep™ Fungi/Yeast Genomic DNA Extraction Mini Kit (Favorgen /Taiwan) was used to extract DNA, according to the steps performed by (12).

PCR conditions for the three regions used

In this study, specific PCR fragment was amplified, partially including ITS1 region, the 5.8 rRNA gene, and the ITS2 region. The reaction was conduct under the following temperature conditions: one cycle of first denaturation at 95°C for 5 minutes; 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 90 seconds; and one cycle of the last extension at 72°C for 5 minutes.

Sequencing methods

PCR sequencing

PCR products were commercially sequenced in both forward and reverse directions by Macrogen Inc. (Geumchon, Seoul, South Korea) following the manufacturer's instructions. To ensure the results were correct, only clear chromatograms extracted from ABI (Applied Biosystem) sequence files were used in subsequent analysis to verify that clarifications and discrepancies were not due to errors in the PCR reaction or the sequencing process. The sequences retrieved from the nucleic acids were compared with the sequences found in local samples, and the possible locations and additional details about the PCR fragments were identified.

Sequence data interpretation

The PCR sequence results of the target samples were modified, aligned, and evaluated using the BioEdit Sequence Alignment Editor (version 7.1), along with the matching sequences in the reference database. The differences identified in each sequenced sample were numbered at two corresponding positions within the PCR products of the reference genome. The positions of the detected nucleic acids were precisely identified and numbered within the PCR products and the reference genome according to the appropriate locations.

Depositing sequences in GenBank

The two sequences that were screened and evaluated were uploaded to the NCBI BankIt portal, adhering to all the guidelines provided by the server (17,18). To obtain a unique accession number (GenBank Accession Number) for the sequences under study, they were submitted as nucleic acid sequences in the NCBI database.

Statistical Analysis

Chi-square tests and ANOVA with LSD post-hoc analysis were performed to assess associations between infection types, age groups, gender, and residence. A $P < 0.05$ was taken into considered.

Results

According to Figure 1, the epidemiological assessment of the 258 confirmed dermatophyte cases demonstrated that Tinea corporis was the most common clinical form, representing 37.89% of all infections. This was followed by T. pedis (16.69%) and T. capitis (15.09%), indicating that trunk and foot infections constituted the majority of dermatophytosis presentations in the study population.

Marked gender-associated differences were observed. Among females, the highest prevalence was recorded for T. corporis (63.9%), T. unguium (92%), T. cruris (92%), T. faciei (65.9%), and T. manuum (81.5%). In contrast, males exhibited a predominance of T. pedis (85.6%) and T. capitis (62.3%), suggesting lifestyle- and exposure-related factors contributing to these patterns (Figure 2).

Analysis of residence-based distribution revealed a higher burden among individuals from rural areas (57.4%), compared to urban residents (42.6%), reflecting potential environmental, socioeconomic, and hygiene-associated determinants.

Age-specific distribution indicated that the > 13 years' age group accounted for the majority of cases (73.5%), while younger age groups exhibited substantially lower infection frequencies

For the first time in Babylon Province, three species belong to *Trichophyton indotineae.* Their colony morphology appeared as white-colored colonies with a cottony to powdery texture on PDA. The colonies exhibited radial growth with irregular margins and dense mycelial development at the center. Microscopic examination revealed the presence of septate, spindle-shaped macroconidia, small oval microconidia, and spiral (helical) hyphae (Figure 3).

Three samples were included in this study within the target site, and these were screened for partial amplification of the ribosomal sequences of the fungi under investigation (Figure 4). This is because the ribosomal sequences can be used for genotyping due to its possible ability to adapt to variable genetic diversity. For the ribosomal amplicons of isolates S1, S2, and S3,

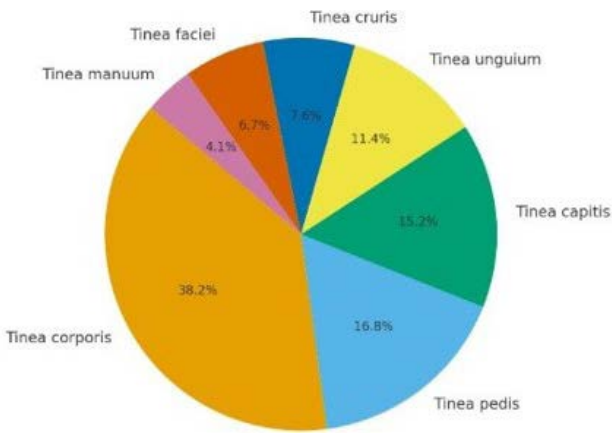


Figure 1. Distribution of dermatophyte infections by type

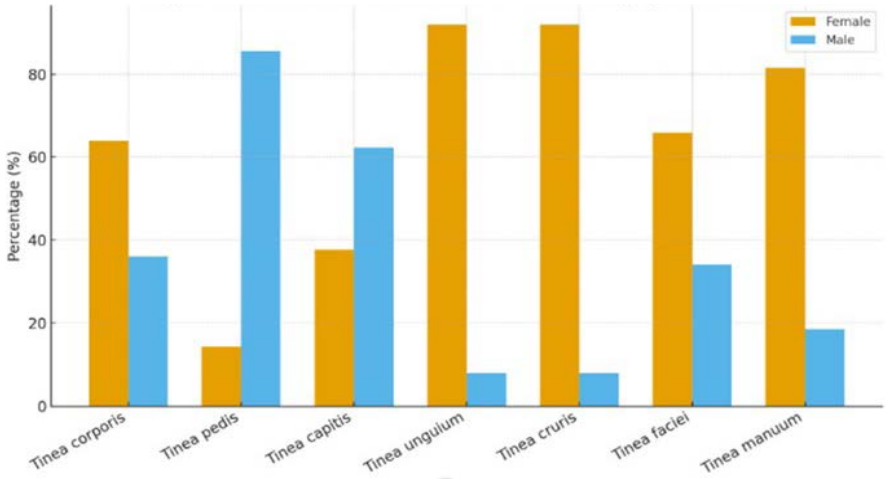
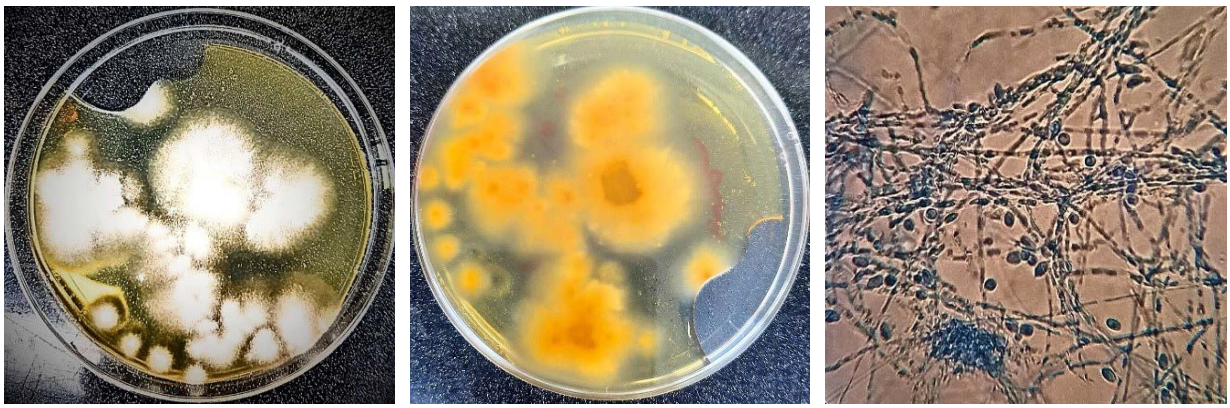


Figure 2. Gender-based distribution of infection types



(A) (B) (C)

Figure 3 Morphological identification of *Trichophyton indotineae*;(A-B) Colony morphology of the fungal isolate grown on potato dextrose agar (PDA) showing white cottony colonies with radial growth and irregular margins. (C) Microscopic morphology showing Fungal hyphae and small oval microconidia

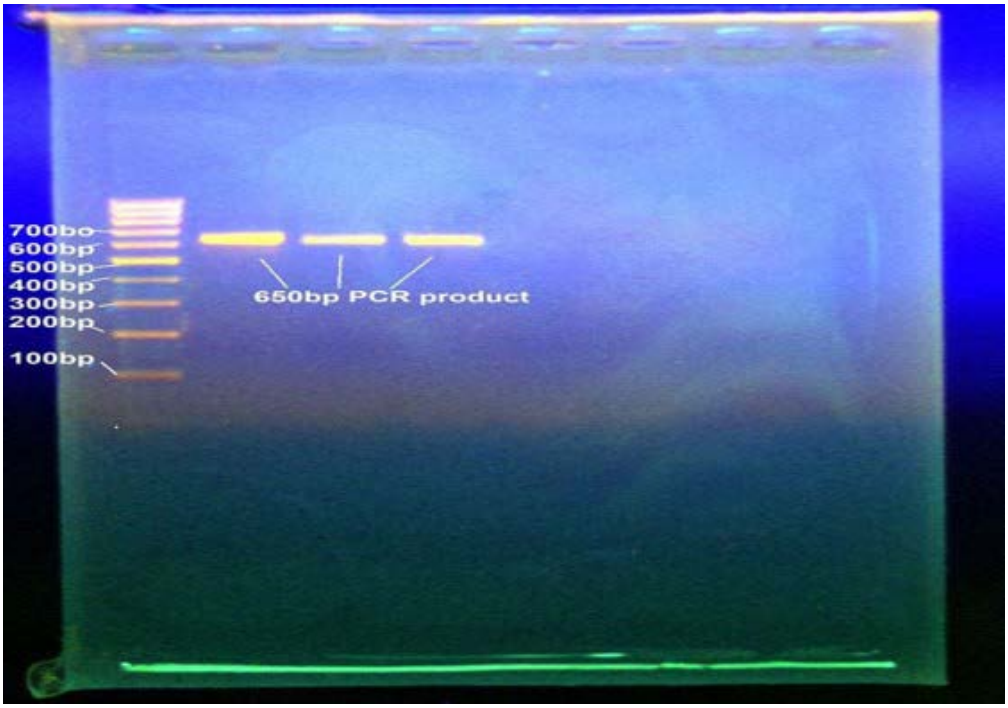


Figure 4. Agarose gel electrophoresis showing PCR amplification of the ITS region of three fungal isolates. Lane M: DNA ladder (1000-100 bp); Lanes 1–3: fungal isolates showing amplification products of approximately 650 bp

the NCBI BLASTn tool showed perfect match between the sequenced samples and the reference sequences. By looking at the nucleic acid sequences in these samples and comparing them with the sequences found in the GenBank database (accession number: ON528187.1), the exact locations and other details of the retrieved PCR segments were determined. The total length of the target site was found on the NCBI server, and the start and end positions of this site were confirmed within the sequence most homologous to *Trichophyton indotineae* (Figure 5).

After the alignment of ribosomal amplicons' sequences with the corresponding fungal genomic regions, the details of its sequences were identified, and the total length of the amplicons was determined (Table 1).

Interestingly, alignment of the ribosomal samples from *T. indotineae* showed no nucleotides variation when compared with the most similar reference sequences (GenBank accession no. ON528187.1) (Figure 6).

Our findings indicated no detectable variations in the nucleotide sequences of fungal samples. To validate this, the sequencing chromatograms and their detailed annotations were thoroughly examined and documented. Additionally, the chromatograms exhibited distinct peaks across nearly all sequencing regions. (Figure 7).

The studied samples were deposited in the NCBI database, and unique accession numbers were assigned to the analyzed sequences. In the present investigation, MEGA 7 (v. 7.2) was employed to infer the phylogenetic relationship, and the trees were constructed using neighbor-joining (19). The comprehensive tree comprised 32 aligned nucleotide sequences Figure 8.

Discussion

As seen in Figure (2) the present study shows a clear predominance of dermatophyte infections among females compared to males. This trend may be attributed to several gender-related risk factors, including more frequent engagement in cosmetic and grooming practices such as manicures, pedicures, and skin care routines, which can disrupt the skin and nail barrier and increase fungal susceptibility. In addition, women often experience greater exposure to wet household environments such as washing, cleaning, and handling damp fabrics which promotes fungal transmission. Differences in health-seeking behavior may also contribute, as females are generally more likely to seek medical care for skin and nail disorders, leading to higher confirmed case detection. Similar patterns have been reported in recent epidemiological studies (15, 5).

This study provides the first documented molecular confirmation of *Trichophyton indotineae* in Babylon Province, Iraq. Epidemiological findings align with global trends where Tinea corporis remains the most common dermatophyte infection. The higher prevalence among females may reflect increased exposure to wet work, cosmetic practices, and healthcare-seeking behavior. Similar gender related trends were reported by (15, 5). These results could be attributed to males' greater involvement in outdoor activities, such as farming, studying, and shepherding, compared to females (11, 14). Otherwise, the current study's findings were incompatible with those of (2), who found that the frequency of dermatophytosis was higher in females (67.05%) than in males (32.94%) in Tunisia. Tinea pedis infection is more common in females than in males, which is the inverse of the situation in the other forms of injury. The reason may be that ladies wear socks more than men, as well as the narrow high-heeled shoes that induce pressure on the foot, sweating, and dampness, which produce an ideal habitat for growth. The most prevalent of dermatophytes with current results age groups more than 10years. This is since in these age groups and because of the thinness of their skin, they become infected through contact with sources of pathogenic fungi, which include infected colleagues, or through pets such as dogs, cats, sheep, horses, pigs, and hedgehogs, especially since children are curious and do not care about their results, which results in them becoming infected with the fungus. It is known that the immune system for children is less effective than in adults (16).

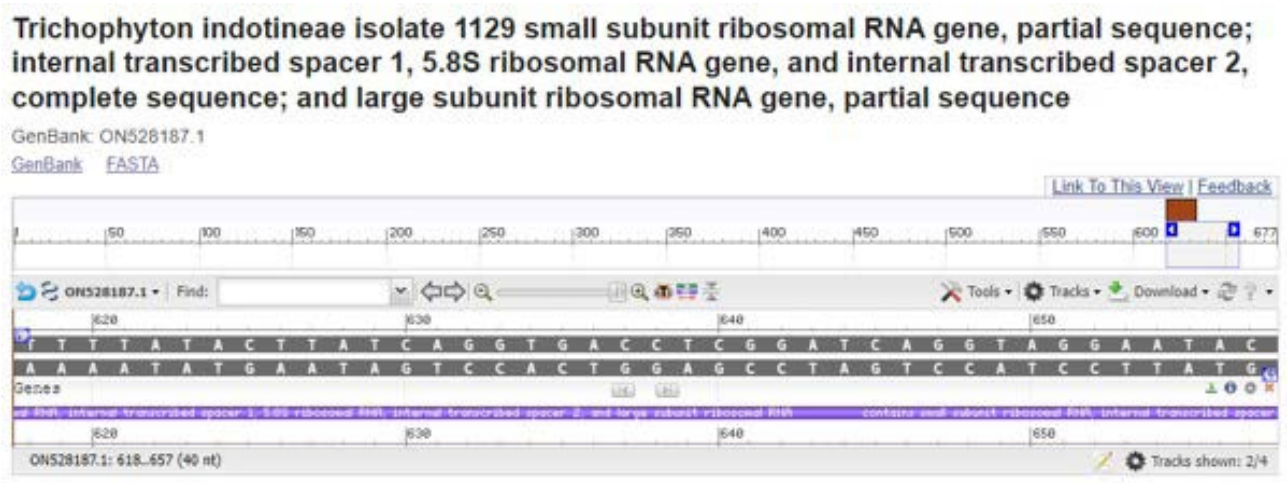


Figure 5. The position of PCR amplicon partially covered the ITS1, 5.8S, and ITS2 regions of the rRNA gene within variable fungal genomic sequences (GenBank accession no. ON528187.1)

Table 1. The position and length of the PCR amplicons that are used to partially amplify the ITS1, 5.8S, and ITS2 ribosomal sequences within the amplified fungal genomic sequences (GenBank accession no. ON528187.1).

Amplicon	Reference locus sequences (5′ - 3′)	length
Ribosomal sequences of the *Trichophyton indotineae*	ACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAACGCGCAGGCCGGAGGCTGGCCCCCCACGATAGG-GCCAAACGTCCGTCAGGGGTGAGCAGATGTGCGCCGGCCGTACCGCCCCATTCTTGTCTACCTTACTCGGTTG-CCTCGGCGGGCCGCGCTCTTCCAGGAGAGCCGTTCGGCGAGCCTCTCTTTAGTGGCTAAACGCTGGACCGCGC-CCGCCGGAGGACAGACGCAAAAAAATTCTTTCAGAAGAGCTGTCAGTCTGAGCGTTAGCAAGCAAAAATCAGTTA-AAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG-CAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGGCATGCCTGTTCGAGC-GTCATTTCAGCCCCTCAAGCCCGGCTTGTGTGATGGACGACCGTCCGGCGCCCCCGTTTTTGGGGGTGCGGGACG-CGCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGCTTCCTAGGCGAATGGGCAACAAACCAGCGCCTCCAGGAC-CGGCCGCCCTGGCCTCAAAATCTGTTTTATACTTATCAGGTGACCTCGGATCAGGTA	650 bp

Figure 6. Sequence alignment of three fungal samples against reference sequences from fungal ribosomal genomic data. The symbol "ref" denotes the reference sequence from NCBI (GenBank accession number ON528187.1), while the notation "S#" represents the sample number

Molecular results underscore the necessity of sequencing-based identification due to the close morphological similarity within the *T. mentagrophytes/T. interdigitale* complex. The 100% ITS identity observed suggests recent introduction or limited evolutionary divergence of *T. indotineae* in Iraq. Phylogenetic clustering of isolates from Europe and South America may indicate international transmission routes associated with travel, migration, or the introduction of fomites.

Terbinafine resistance in *T. indotineae* has been widely (3). Although antifungal susceptibility testing was not performed in this study, the detection of genetically conserved strains highlights the importance of including susceptibility profiling in future work.

Phylogenetic analysis of *Trichophyton* isolates is considered one of the most exciting findings analysis of *Trichophyton* isolates that, identification of our samples within different evolutionary lineages within this genus. This is because ribosomal sequence variation can be used for genotyping due to its ability to adapt to changing genetic diversity. Sequencing reactions confirmed complete congruence after performing NCBI BLASTn on the PCR products (10, 15). Because of its ability to cause significant variation in its position within the resulting lineages, the detected nucleic acid sequences were shown to have evolutionary implications that indicate this type of diversity. As a result, these differences led to clear distinctions within the same *Trichophyton* species. Within the phylogeny of *T. indotineae*, samples S1, S2, and S3 were found to be close to two strains isolated from Italy

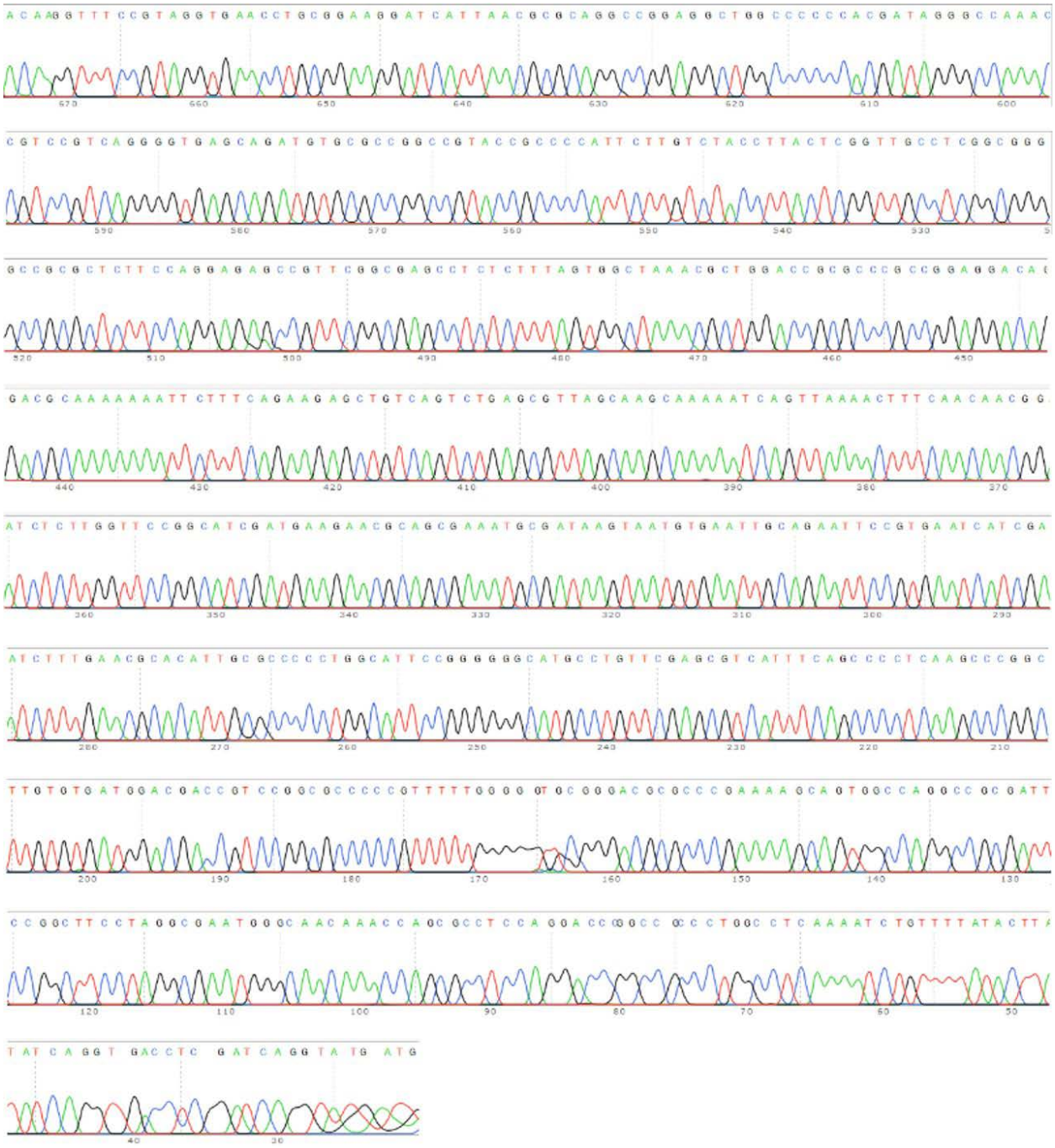


Figure 7. Chromatogram of the investigated fungal sequences amplified by PCR

and Argentina respectively, registered in the GenBank database under the codes OR192943.1 and OQ975444.1.

Sample sources and distribution of *T. indotineae* the multiplicity of sample sources analyzed has been confirmed. In a recent study, (16, 20) four new cases of T. cruris and tinea corporis caused by *T. indotineae* were detected in northern Italy. Since the first nucleotide sequence of this fungus from a human skin sample was published in GenBank in 2004 (accession number AB430471.1), *T. indotineae* has been identified in India. In addition, the resistance of *T. indotineae* to terbinafine has increased to 75%, compared to 44% for *T. rubrum*, due to the inappropriate use of corticosteroids, antibiotics, and antifungals (5, 11, and 21). Other potential factors for spreading *T. indotineae* infection in developing countries include working conditions in hot and humid environments with poor hygiene, wearing tight synthetic clothing, and living in crowded conditions (9, 22). The impact of migration and tourism on the spread of *T. indotineae*. The large-scale migrations and the restoration of tourism activity after the COVID-19 pandemic may have contributed to the transmission of several pathogens, in particular *T. indotineae*, which should not be underestimated. Moreover, the anthropophilic nature of this fungus facilitates its transmission between individuals, with studies showing that 50% of patients were infected within a family setting, with particular reference to the role of contaminated personal items (fomites) in

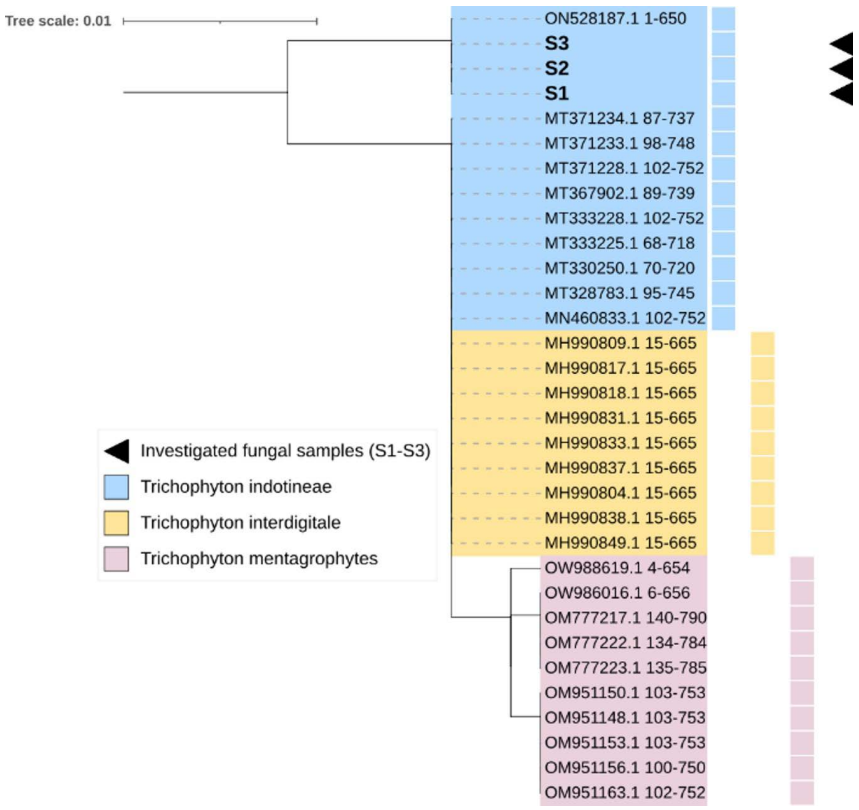


Figure 8. Phylogenetic tree of ribosomal sequences for *T. indotineae* is represented in a rectangular cladogram (Branch A) and a circular cladogram (Branch B). The black triangle indicates the analyzed samples. All numerical references correspond to the GenBank accession numbers for the respective species. The number at the top of the tree denotes the scale range among the categorized organisms. The notation "S#" represents the code assigned to the investigated samples

the transmission of infection (13, 23). Corticosteroids were first used in clinical practice in 1949 to treat rheumatoid arthritis, and since then, their applications have expanded to include many organs and diseases, including dermatology (1, 2, 6). The sequencing reactions revealed the precise identity of these PCR amplicons after using NCBI BLASTn (19, 24).

Conclusions

The molecular confirmation of *Trichophyton indotineae* in Babylon Province marks a critical epidemiological shift in Iraq's dermatological landscape. Because *T. indotineae* is globally recognized for its high rate of genomic resistance to terbinafine, its local emergence necessitates an immediate transition from empirical antifungal prescribing to laboratory-guided stewardship. Public health strategies must prioritize the integration of routine molecular diagnostics and localized antifungal susceptibility testing to prevent widespread treatment failures. Furthermore, the higher burden observed in rural cohorts and specific demographic groups underscores the need for targeted hygiene education, regulated access to over-the-counter topical corticosteroids, and active genomic surveillance to track potential transmission routes and curb the dissemination of this refractory pathogen.

Acknowledgments

The authors thanks Baghdad and Mustansiriyah Universities for their support in the current work.

Authors' Contribution

Conceptualization: Faheema Jabbar, Sinai Waleed Mohammed, Dina Yousif Mohammed
Data curation: Huda Mushtaq Talib, Mervet Abdul Hasan Mshachal
Formal analysis: Huda Mushtaq Talib, Mervet Abdul Hasan Mshachal
Funding acquisition: Sinai Waleed Mohammed
Investigation: Zaman Abdul Hussein Ali Ghfil, Dina Yousif Mohammed
Methodology: Dina Yousif Mohammed, Faheema Jabbar Aboalhur, Sinai W. Mohammed
Project administration: Sinai Waleed Mohammed, Ahmad Yosif Hanoon
Resources: Huda Mushtaq Talib, Mervet Abdul Hasan Mshachal
Software: Faheema Jabbar, Sinai Waleed Mohammed
Supervision: Dina Yousif Mohammed, Faheema Jabbar Aboalhur, Sinai W. Mohammed
Validation: Faheema Jabbar, Sinai Waleed Mohammed
Visualization: Faheema Jabbar, Sinai Waleed Mohammed
Writing – original draft: Faheema Jabbar, Sinai Waleed Mohammed
Writing – review & editing: Dina Yousif Mohammed, Faheema Jabbar Aboalhur, Sinai W. Mohammed

Competing Interests

The authors declare no competing interests.

Ethical Approval

The ethical committee of the College of Science Research accepted this study (CSEC/0225/0022) on April 12, 2025.

Funding

Nil.

Data Availability Statement

The datasets used and analyzed during the current study are

available from the corresponding author on reasonable request.

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