

Genetic Evolutionary Profile of Dermatophytes Isolated from Tinea Capitis Patients in Iraq

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Abstract

Objective: Tinea capitis remains a significant public health challenge worldwide, particularly among pediatric populations in developing regions. This study was designed to investigate the genetic diversity, species distribution, and evolutionary relationships of *Trichophyton* spp. isolated from tinea capitis patients.

Methods: Fifty-four clinical specimens (skin scrapings and hair fragments) were collected from outpatients presenting with symptomatic Tinea capitis. The specimens were subjected to direct microscopic examination and fungal culture for the isolation and identification of the causative fungal agent at the species level using both phenotypic and molecular approaches. Molecular identification was performed through sequencing of the amplified Internal Transcribed Spacer (ITS) region. Genetic and evolutionary relationships among *Trichophyton* species were analyzed by constructing phylogenetic trees using MEGA 12.0. *M. canis* was identified phenotypically and was not included in the ITS-based phylogenetic analysis.

Results: Phenotypic and molecular profiling of the examined fungal pathogens revealed that the *Microsporum canis* was the most frequently identified species, followed by *T. tonsurans*, *T. mentagrophytes*, and *T. verrucosum*. ITS-based phylogenetic analysis was performed only for the 17 *Trichophyton* isolates. The total identified isolates were 54 included *T. mentagrophytes* ($n = 6$, 11.11%), *T. verrucosum* ($n = 4$, 7.41%), *T. tonsurans* ($n = 7$, 12.96%) and *M. canis* ($n = 37$, 68.52%). Intraspecies genetic evolutionary relatedness, deciphered from three phylogenetic trees constructed based on the DNA sequence of the Internal Transcribed Spacer region, was assessed only for the three species of *Trichophyton* (17 isolates): *T. mentagrophytes*, *T. verrucosum*, and *T. Tonsurans*. The strains belonging to each species clustered into distinct clades.

Conclusion: The ITS-based phylogenetic analysis demonstrated genetic heterogeneity among Iraqi *Trichophyton* isolates. These findings suggest the circulation of multiple genetically diverse *Trichophyton* lineages among tinea capitis patients in Iraq.

Keywords: Tinea Capitis, dermatophytes, Internal Transcribed Spacer (ITS), DNA sequencing

Introduction

On a worldwide scale, superficial dermatophytosis (ringworms, tinea) represents a significant health concern associated with considerable health and economic burdens. Recently, in 2024, rough estimates reported by the Center for Disease Control and Prevention (CDC) and the World Health Organization (WHO) indicated that approximately millions of patients of fungal skin infections worldwide are attributed to ringworms. Tinea, commonly referred to as ringworms, affects more than 25% of school-aged children, many of whom suffer from scalp skin and hair shafts infections known as (Tinea Capitis).¹⁻³ Ringworms and fungal pathogens are highly transmissible that can infect both humans and animals. Transmission of ringworms occurs through direct contact with infected animals or humans, as well as through exposure to contaminated soil. In view of that, dermatophytes may be classified as anthropophilic (human-adapted) or zoonotic (animal origin) or geophilic (soil-dwelling) pathogens.⁴

Tinea capitis, a dermatophytosis of the scalp and hair follicles, displays a wide variety of clinical presentations, including both inflammatory and non-inflammatory forms. The non-inflammatory forms of tinea capitis are characterized by diffuse scaling, gray patches, and black dots lesions, whereas the inflammatory forms encompass diffuse pustular lesions and kerion.^{5,6} Tinea capitis is more prevalent among children at the age of 3–14 years, with a decreased incidence upon puberty due to maturation of the immune system and the antifungal activity of fatty acids in postpubertal sebum.

Currently, Tinea capitis exhibits an alteration of the prevalence and incidence among adults with immunosuppression, hormonal disturbance (particularly women at post menopause), and systemic chronic diseases.^{6,7} The key etiological agent of Tinea capitis are dermatophytes belonging to the genus *Trichophyton*, including *T. rubrum*, *T. mentagrophytes*, *T. verrucosum*, *T. violaceum*, among others.⁸⁻¹⁰

Due to the limitations associated with traditional phenotypic methods for speciation of the fungal causative agent, molecular approaches, particularly sequencing of the internal transcribed spacer region, have become the gold standard approach for definitive speciation.^{11,12} In the light of the aforementioned consideration, the prevalence of Tinea capitis in Iraq remains unclear. Therefore, the present study seeks to unravel the prevalence of ringworms species among Iraqi tinea capitis patients via phenotypic approaches (cultural and microscopic) alongside molecular-based approach (ITS region sequencing) and explore the genetic diversity among the dermatophytes isolates.

Methods

Study Cohorts, Ethical Approval, Data Collection

Fifty-four patients clinically diagnosed with clinical presentation with Tinea capitis (Figure 1) and attending the Dermatology Departments of Al-Zahraa Teaching Hospital, Al-Karama Teaching Hospital, and Al-Nu'maniya General Hospital, Wasit Province were enrolled in this study. Ethical

approval was obtained from the Institutional Review Board of the Scientific Committee, of College of Medicine, University of Al-Qadisiyah, Iraq (Reference No. 48, October 2024) prior to the collection of clinical specimens. All study procedures were conducted in strict accordance with the ethical principles of Declaration of Helsinki. A written informed consent was obtained from all participants before specimens and data collection. In addition, all participant data were anonymized to ensure privacy and confidentiality.

Information regarding the dynamics of dermatophytes transmission was systematically collected from all patients with Tinea capitis through standardized questionnaires and clinical interviews conducted at the time of specimen collection. The collected data included a detailed evaluation of clinical history to recognize patterns of primary contact, explicitly regarded as direct patient-to-patient contact (i.e., anthrophilic transmission) or direct contact with infected animals (i.e., zoophilic transmission).

Collection of Clinical Specimens

The clinical specimens were collected from December 2024 to June 2025 according to established procedures.¹³ Each patient was subjected to a strict sampling procedure, where hair shafts were plucked from the affected area using sterile forceps to help ensure that the hair root was considered (Figure 1). Further, sufficient skin scalp scrapings from the active periphery of the lesion were collected using sterile scalpel or the edge of a glass microscopic slide.

Diagnosing Tinea Capitis: Isolation and Identification

(a) Microscopic examination of dermatophytes hair infection

The infected hair shafts were treated with 10–20% KOH according to a previously reported procedure.¹⁴ Then the treated specimens were examined under the light microscope.

(b) Isolation and Culturing of the dermatophytes

The clinical specimens exhibited positive microscopic examination after KOH were inoculated onto the surface of Sabouraud's Dextrose Agar supplemented with 1g/L. as saprophytic fungi and bacterial growth suppressor. Once confirmed, the clinical material is inoculated onto a primary isolation medium, typically Sabouraud's Dextrose Agar (SDA) (Himedia Co., India) supplemented with

cycloheximide and chloramphenicol at a concentration of 1 mg/L and 0.05 g/L, respectively to suppress the growth of saprophytic fungi and bacteria. The inoculated SDA plates were incubated at 25–30 °C for one to four weeks in incubator (Constant Temperature Incubator, BJPX-HII, BIOBASE Co., China).

Dermatophytes suspected colonies raised on SDA plates were sub-cultured onto Dermatophytes Test Medium (Becton, Dickinson (BD, Sparks, MD, USA)). The DTM medium contained a phenol red indicator detecting the alkaline metabolites produced by dermatophytes and three antimicrobial agents (cycloheximide, chloramphenicol, and gentamicin at concentrations of 0.5, 0.1, and 0.1 g/L, respectively).¹⁵ The inoculated DTM plates were incubated at 25–30 °C for two weeks in incubator (Constant Temperature Incubator, BJPX-HII, BIOBASE Co., China).

(c) Internal transcribed spacer (ITS) region amplification and sequence similarity search analysis:

-Fungal genomic DNA isolation

Only 17 *Trichophyton* isolates (*T. mentaephytes* ($n = 6$), *T. verrucosum* ($n = 4$), *T. tonsurans* ($n = 7$), presumptively identified based on microscopic and cultural features were subjected to DNA isolation using PicoPure™ DNA Extraction Kit (Applied Biosystems™, Thermo Fisher Scientific Co., USA) according to the manufacturer's instructions". The quality and quantity of the isolated DNA were assessed via Nano-drop spectrophotometer measurements (NanoDrop™ 8000 Spectrophotometer, Thermo Fisher Scientific Co., USA).

Amplification of Internal Transcribed Spacer (ITS) Region

The internal transcribed spacer (ITS) region from the suspected *Trichophyton* isolates was amplified by polymerase chain reaction (PCR) using dermatophytes specific ITS specific primer set: Forward: LR1 5'-GGTTGGTTTCT-TTTCCT-3' and Reverse: SR6R 5'-AAGTAAAGTCGTAA-CAAGG-3' amplifying a fragment of 630–700 bp.¹⁶ The PCR reaction mixture included 0.5 µg of genomic DNA, 1.5 µM of each primer, and 25 µL of PCR Master Mix (2X) (Thermo Fisher Scientific™, Co. USA), adjusted to a final volume of 50 µL using nuclease-free water. The thermocycler conditions were set as follow: 95°C, 5 min (initial denaturation, one cycle), (30 cycles; each 94°C, 30 sec for denaturation, 52°C, 30 sec for annealing, and 72°C, 30 sec for extension), and final extension



Fig. 1 Representative of cases clinically diagnosed with dermatophytosis (Tinea capitis) showing ring worm of scalp infection by *Trichophyton* species.

(72°C, 5 min). After PCR termination, an aliquot of PCR was checked on 1% agarose gel electrophoresis.¹⁷

Statistical Analysis

Sequences were aligned via ClustalW and constructed Neighbor-Joining or Maximum Likelihood phylogenetic trees using software like MEGA version 12.0 to deduce structural clades and species distribution.

Results

Presumptive Identification of Dermatophytes Isolated from *Tinea Capitis* Patients

Microscopic examination revealed that the pre-treated infected hair fragments with 10–20% KOH yielded a dense, interconnected network of dermatophytes structures with infiltration to the keratin host tissue as depicted in Figure 2.

Abundant septate, hyaline, and ramose-arthroconidia forming fungal hyphae in the lysed epithelial fields were observed. These characteristic microscopic findings in the infected hair fragments were consistent with dermatophyte infection as the causative agent of *Tinea capitis*. All fifty-four clinical specimens yielded positive direct findings for dermatophytes following treatment with 10–20% KOH. Consequently, all fifty-four clinical specimens were subjected to culturing onto Sabouraud Dextrose Agar (SDA) plates. Prolonged incubation of suspected dermatophyte colonies on SDA plates for more than four weeks provided additional insights into their colonial morphological features as depicted in Figure 3. Suspected *T. mentagrophytes* exhibited a rapidly expanding, flat, creamy floccose macro-colonies with a characteristic yellowish-tan to brownish reverse (Figure 3). Furthermore, another suspected species of the genus *Trichophyton* namely *T. tonsurans* showed a highly pleomorphic, flat white colony with a notably dark, concerted mahogany-brown to yellowish reverse midpoint (Figure 3). In addition, suspected

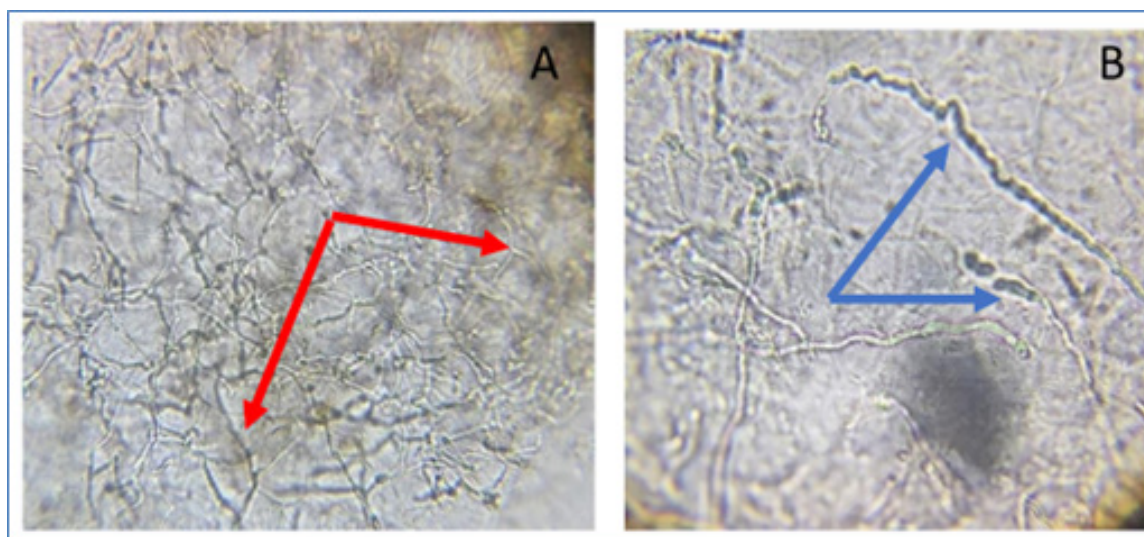


Fig. 2 A light microscopic slide field (40X magnification) demonstrating the fungal hyphae filaments and conidia of the dermatophyte, *Trichophyton* spp. incorporated in KOH treated infected hair mounts. A): fungal hyphae pin pointed indicated with red arrows. B): fungal conidia pin pointed indicated by blue arrows.

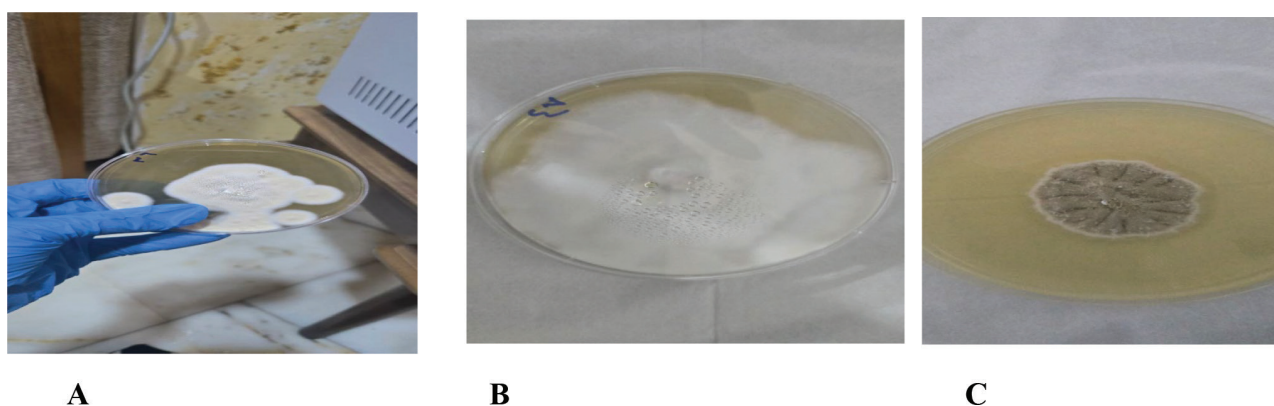


Fig. 3 Colonial distinctive features of *Trichophyton* spp. upon extended incubation on SDA plates beyond 4 weeks at 28°C. The suspicious dermatophytes are *T. mentagrophytes* (A), *T. tonsurans* (B), and *T. verrucosum* (C).

T. verrucosum isolates demonstrated slow-growing, glabrous-to-waxy gray-white button colony with a submerged, dark-tan wrinkled reverse (Figure 3).

Data derived from microscopic examination of infected hair shafts treated with 10–20% KOH and colonial morphological features on both SDA plates presumptively suggested that the suspect fungal pathogen in the clinical specimens.

Confirmatory Identification of Dermatophytes Species Via ITS Region Sequencing

The ITS region from 17 *Trichophyton* spp. isolates were successfully amplified using ITS specific primer set, with a partial fragment of 700 bp (Figure 4).

The ITS nucleotide sequences of the six suspected *T. mentagrophytes* were identified as *T. mentagrophytes*, showing sequence identities of 99.18–99.84% and a query coverage of 100% when compared with the reference sequence MN886819.1 (*T. mentagrophytes* 250036118). The ITS nucleotide sequences of *T. mentagrophytes* isolated were submitted in the GenBank under the accession numbers PZ448038.1 to PZ448043.1 and the isolates were denoted nomenclature *T. mentagrophytes* BAQMM38 to *T. mentagrophytes* BAQMM43. The ITS nucleotide sequences of four suspected *T. verrucosum* were affiliated as *T. verrucosum* with identity and query coverage of 99.5–99.84% and 100%, respectively to subject sequence of MN37920.1 (*T. verrucosum* MASR 1976c). These ITS nucleotide sequences were deposited in the GenBank under the accession numbers of PZ448051.1 to PZ448054.1, while the isolates were designated as *T. verrucosum* BAQMM51 to *T. verrucosum* BAQMM54. Finally, the ITS nucleotide sequences of suspect *T. tonsurans* were affiliated to *T. tonsurans* with identity and query coverage of 99.11–99.72% and 100%, respectively to subject sequence (MN37920) *T. tonsurans* BMU02135. *T. tonsurans* isolates were assigned strains designation as *T. tonsurans* isolate BAQMM44–*T. tonsurans* isolate BAQMM49. The ITS nucleotide sequences were deposited in the Gen Bank under the accession numbers PZ448044.1 to PZ448050.1.

Phylogeny and Lineages of Clinical *Trichophyton* Isolates

To unravel the genetic relatedness and lineages of clinical *Trichophyton* isolates, three Neighbour-Joining (NJ)

phylogenetic trees (Figure 5) were constructed using MEGA 12.0, where the genetic distances were calculated based on Kimura-2-parameter model and the reliability of the branching profiles was evaluated by performing a bootstrap analysis of 1,000 re-samplings. The ITS-based phylogeny analysis demonstrated genetic heterogeneity among Iraqi *Trichophyton* isolates, with clustering into distinct sequence-based clades. These findings suggest the circulation of multiple genetically diverse *Trichophyton* lineages among tinea capitis patients in Iraq.

Analysis of phylogeny tree (Figure 5B) of Iraqi clinical isolates of *T. tonsurans* revealed the majority of Iraqi isolates were clustered within Clade I, which comprised a mixture of Iraqi clinical isolates (BAQMM 44, 45, 48, and 49) and international *T. tonsurans* isolates originating from China, Turkey, and Belgium. This clustering pattern suggests a close genetic relationship between the Iraqi isolates and geographically diverse international strains. The basal lineage, localized at the very base of the tree, was represented by clade II, which included the Iraqi Clinical *T. tonsurans* isolates namely BAQMM46 and 47. Clade II was separated by a significant distance from the main cluster (Clade I). Therefore, the isolates in clade II harbor distinct variants that may not share the same ancestral history dominant in clade I isolates. A third intermediate lineage was evidenced in the middle of the tree, represented by isolate *T. tonsurans* BAQMM50, which was separated by a substantial distance from the other two clade I and clade II. This would suggest a distinct third lineage. Collectively, there is a greater genetic diversity within the local clinical Iraqi *T. tonsurans* population.

Analysis of phylogenetic tree (Figure 5C) of the local clinical isolates of *T. verrucosum* revealed the distribution of the four isolates into three distinctive clade of different ancestral history and large genetic distances. The primary lineage (Clade I), localized in the top of the tree, encompassed the isolate BAQMM53, exhibiting similarity to other local Iraqi isolate not enrolled in this study. The intermediate lineage was presented by the isolate BAQMM51, exhibiting close proximity to international *T. verrucosum* isolates from Japan, Iran, China, and Russia. Finally, the last lineage was included in clade III (with two clinical Iraqi isolates: BAQMM52 and BAQMM54). The third and last clade showed a far genetic distance compared to the other two lineages, localized in the top and middle of the phylogeny tree. Clade III isolates were genetically close to international *T. verrucosum* from Egypt and Germany.

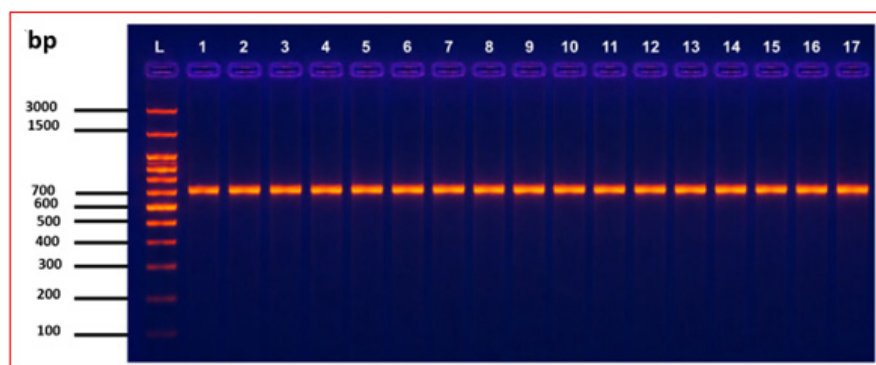


Fig. 4 1% agarose gel electrophoresis showing the amplification of ITS region of 18S ribosomal DNA from 17 *Trichophyton* isolates. Lanes 1–17: PCR products (700 bp). Lane L: DNA ladder.

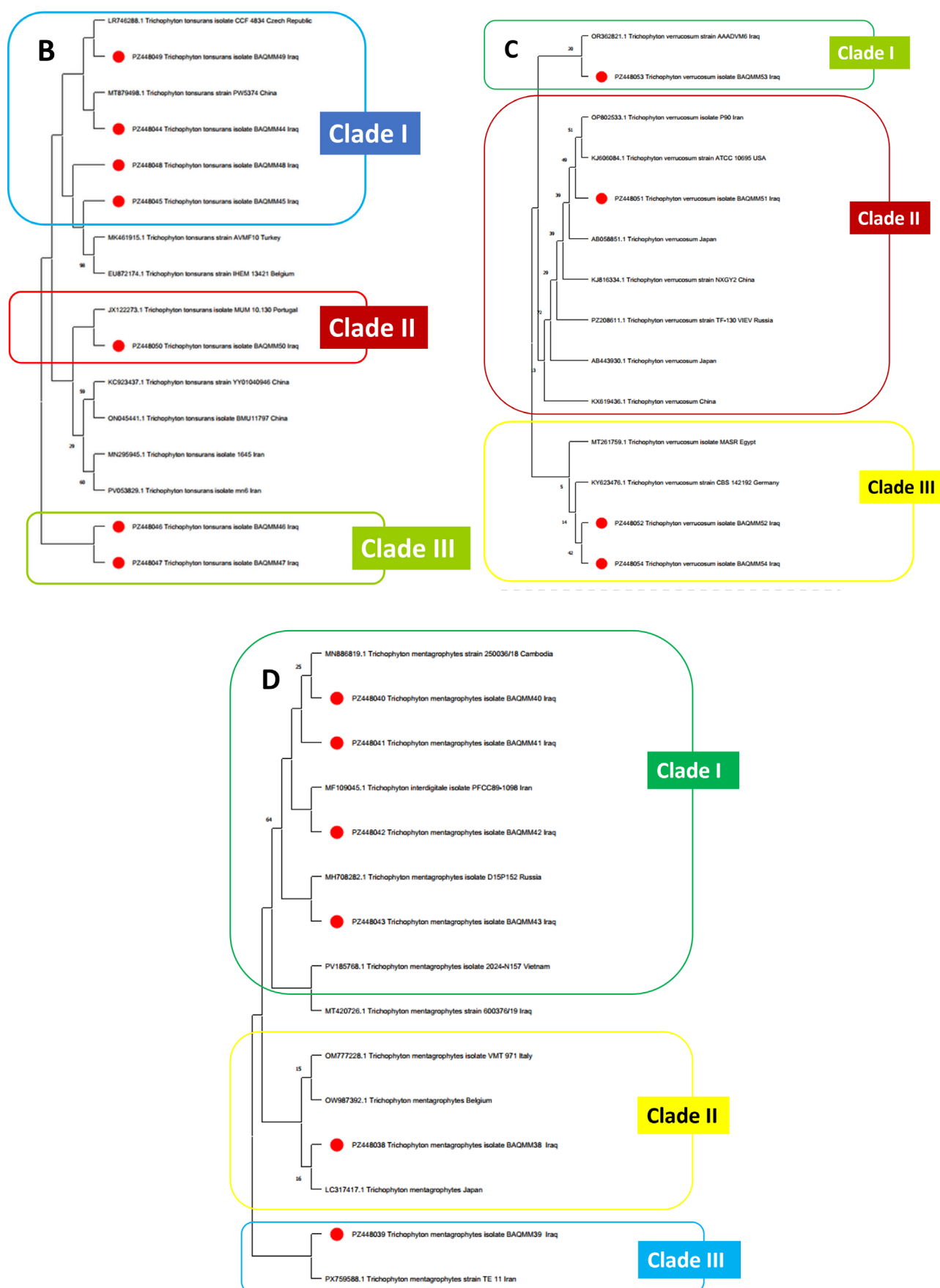


Fig. 5 Neighbor-Joining phylogenetic trees constructed using MEGA 12.0 to illustrate the evolutionary relationships between Iraqi clinical *Trichophyton* isolates and closely related international reference strains. Bootstrap values were calculated from 1,000 replicates. B: seven Iraqi *T. tonsurans* isolates; C: four Iraqi *T. verrucosum* isolates; D: six Iraqi *T. mentagrophytes* isolates.

For *T. mentagrophytes* isolates, analysis of phylogeny tree (Figure 5D) revealed that there was three distinctive clades with three suggested separate lineages: clade I, II, and III. Clade I, in the top of the tree, involved a mix of local Iraqi *T. mentagrophytes* isolates BAQMM40 to BAQMM43 and international *T. mentagrophytes* isolates from Vietnam, Russia, Iran, and Cambodia. Clade II had a distinct variant of local Iraqi *T. mentagrophytes* BAQMM38 with proximity to world-wide *T. mentagrophytes* isolates from Italy, Japan, and Belgium. The third basal lineage was clade III, which had a distinct variant BAQMM39 with proximity to an international isolate from Iran.

Discussion

The preliminary identification of dermatophytes isolates in the present study was achieved through microscopic examination of infected hair shafts following treatment with 10–20% KOH that facilitated the visualization of fungal elements infiltrating keratinized tissues. The observation of septate, hyaline hyphae together with arthroconidia provided a robust clue of dermatophytic involvement. Following this initial assessment, culture-based methods using SDA empower the identification of particular colonial phenotypes affiliated to *Trichophyton* spp. Despite the value of phenotypic methods as fundamental diagnostic approach in clinical laboratories for the identification of etiology of dermatophytes, they have some limitations such as the necessity for a significant expertise, the indispensable demand for prolonged incubation times to get sufficient fungal growth with well-observed textural features and pigmentation, and inability to identify closely related species of dermatophytes. Consequently, molecular-based approaches have emerged as significant complementary tools that provide more accurate method and definite species identification. Such methods enhance diagnostic confidence and support for physician in prescribing the proper medications for patients with *Tinea capitis*.^{18–21}

Numerous studies have addressed the robustness of internal transcribed spacer region in sequencing for the accurate identification and taxonomic classification of dermatophyte species, thereby overcoming many of the limitation associated with conventional-based diagnostic approaches (i.e., culturing methods and microscopic examination of fungal mycelia and conidia). Similarity-based sequence

analysis using BLASTN, has been employed to analyze the highly variable regions ITS1 and ITS2 for specification of the dermatophytes complex species. In the present study, the findings revealed that ITS sequence analysis succeeded not only to unveil the species of the suspicious dermatophyte isolates but also stratify the 17 isolates into numerous clades with genetic evolutionary relatedness. The phylogenetic trees depicted in Figure (5B–5D) conferred the recognized features encountered in ITS sequence analysis alongside phylogenetic analysis to discover at least two distinct lineages in each dermatophyte species: *T. mentagrophytes*, *T. tonsurans*, and *T. verrucosum*. These two distinct lineages deciphered from the three phylogenetic trees depicted in Figure (5B–5D) originate from one common ancestor and divergent subclade. These findings provide significant insights into the molecular epidemiology of the 17 dermatophytes isolates in comparison to other circulating international dermatophytes isolates.^{20–26}

A limitation of this study is that *M. canis* isolates were identified phenotypically and were not included in ITS-based molecular confirmation or phylogenetic analysis.

Conclusion

In conclusion, ITS-based phylogenetic analysis demonstrated genetic heterogeneity among Iraqi *Trichophyton* isolates, suggesting the circulation of multiple genetically diverse *Trichophyton* lineages among patients with *Tinea capitis* in Iraq.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Disclosure of AI-Assisted Writing

The authors declare that no artificial intelligence tools were used in the preparation, writing, or language editing of this manuscript. The authors take full responsibility for the scientific content of the manuscript. ■

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