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Isolation and Identification of Dermatophytes from Toenails and Interdigital Spaces of Students Using Czapek Yeast Extract Agar

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Abstract

Dermatophytosis remains a common superficial fungal infection affecting nails and interdigital spaces, with *Trichophyton rubrum* and *T. mentagrophytes* as the predominant etiologic agents. The recent emergence of terbinafine-resistant *T. indotineae* has raised new challenges in diagnosis and treatment. Although Sabouraud Dextrose Agar is the conventional medium for fungal culture, alternative media such as Czapek Yeast Extract Agar (CYA) may provide additional advantages for dermatophyte isolation and morphological identification. This study aimed to isolate and identify dermatophytes from toenails and interdigital spaces of university students using CYA medium and to evaluate its applicability in dermatophyte culture. A cross-sectional descriptive study was conducted among 100 university students aged 18–24 years. Specimens were collected from toenails (n = 60) and interdigital spaces (n = 40). Direct microscopy was performed using 20% KOH preparation. Samples were cultured on CYA supplemented with chloramphenicol and cycloheximide, and incubated at 28 ± 2 °C for up to 21 days. Fungal isolates were identified based on macroscopic and microscopic morphology. Data were analyzed descriptively, and associations were tested using the chi-square test. Fungal elements were detected in 65% of samples by KOH examination, and dermatophyte growth was confirmed in 52% of cultures. The most frequently isolated species were *T. rubrum* (28%) and *T. mentagrophytes* (18%). Notably, *T. indotineae* was detected in 5% of samples. No significant difference was found between toenail and interdigital isolates (p = 0.26). Dermatophytes are prevalent among university students, with *T. rubrum* as the dominant species. The detection of *T. indotineae* highlights its emerging role in young populations. CYA proved effective for dermatophyte isolation and may serve as an alternative culture medium in academic and diagnostic laboratories.

Keywords: dermatophytes; *Trichophyton rubrum*; *Trichophyton indotineae*; toenail; Czapek Yeast Extract Agar.

Abbreviations: Czapek Yeast Extract Agar (CYA); Potassium Hydroxide (KOH); Lactophenol Cotton Blue (LPCB); Sabouraud Dextrose Agar (SDA); Statistical Package for the Social Sciences (SPSS).

INTRODUCTION

Dermatophytes are a group of keratinophilic fungi belonging to the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. They invade keratinized tissues such as skin, hair, and nails, leading to infections collectively known as dermatophytosis (Gupta, Venkataraman, & Hall, 2023). Among these, toenail onychomycosis and tinea pedis in the interdigital spaces are the most common clinical forms, frequently associated with occlusive footwear, humidity, and communal living environments (Lee, Park, & Kim, 2023).

Globally, onychomycosis remains a significant public health concern because of its chronic course, recurrence, and therapeutic challenges. The condition is most often caused by *Trichophyton rubrum* and *T. mentagrophytes*, with prevalence rates ranging between 10–30% depending on the population studied (Gupta, Versteeg, & Shear, 2022). Recent years have also seen the emergence of *Trichophyton indotineae*, a newly characterized species responsible for difficult-to-treat dermatophytosis. This organism shows a high rate of terbinafine resistance due to mutations in the squalene epoxidase gene, and has now been reported in Asia, Europe, and North America (Chowdhary et al., 2022; McTaggart et al., 2025; Marbaniang et al., 2025). Reports from the United States and Canada further highlight the increasing spread of terbinafine-resistant dermatophytes, raising concerns about limited therapeutic options (Caplan et al., 2023; Fuller, Hay, & Arenas, 2024).

Diagnosis of dermatophytosis is traditionally based on direct microscopy and fungal culture. While fungal culture remains the gold standard, it is limited by low sensitivity and extended incubation periods, which can

delay clinical decision-making (Zhou, Li, & Wang, 2022). Conventional culture media such as Sabouraud Dextrose Agar (SDA) are commonly used, but alternative formulations may improve isolation rates and morphological characterization. Czapek Yeast Extract Agar (CYA), widely applied for *Aspergillus* and *Penicillium*, contains sucrose and yeast extract that can also support dermatophyte growth and provide distinctive colony morphology (Ahmadi et al., 2012; TM-Media, n.d.). Recent reports suggest its potential value for research and diagnostic purposes when studying dermatophytes (Kumar, Patel, & Singh, 2025).

Despite extensive studies on dermatophytes globally, there is limited data on their prevalence in young adults, particularly among university students at risk due to lifestyle and environmental factors. Furthermore, there has been little exploration of CYA as an alternative medium for dermatophyte isolation in this context.

Therefore, this study aimed to isolate and identify dermatophytes from toenails and interdigital spaces of university students using Czapek Yeast Extract Agar. The results are expected to provide baseline data on dermatophyte prevalence in young populations and to evaluate the applicability of CYA as a culture medium for dermatophyte identification.

MATERIALS AND METHODS

Study Design and Population

This was a cross-sectional descriptive study conducted from August 2024 to April 2025 at the Mycology Laboratory, Kadiri University. The study population consisted of undergraduate students from Faculty of Health Sciences, Kadiri University, aged 18–25 years. Inclusion criteria were students who provided informed consent and had no systemic antifungal treatment in the past three months. Exclusion criteria were students with systemic immunodeficiency, current systemic antifungal therapy, or nail trauma unrelated to fungal infection.

Sample Collection

Specimens were collected from toenails suspected of onychomycosis and from interdigital spaces showing scaling, maceration, or erythema. Nail samples were obtained by clipping the distal portion of the affected nail and scraping subungual debris with a sterile scalpel. Interdigital scrapings were collected using a sterile scalpel blade. All specimens were placed into sterile paper envelopes and transported to the laboratory within 2 hours of collection.

Direct Microscopy

Each sample was subjected to a 20% potassium hydroxide (KOH) wet mount preparation. The preparations were examined under light microscopy at 10× and 40× magnification for hyaline septate hyphae or arthroconidia suggestive dermatophyte infection.

Culture and Isolation

Samples were inoculated onto Czapek Yeast Extract Agar (CYA) (TM Media, India; TM-2049) supplemented with chloramphenicol (50 mg/L) and cycloheximide (500 mg/L) to inhibit bacterial and saprophytic fungal contaminants. Inoculated plates were incubated at 28 ± 2 °C for up to 21 days and observed every 3–4 days. Colony morphology—including texture, pigmentation, and growth rate—was recorded.

Identification of Fungi

Macroscopic identification was based on colony morphology and pigmentation. Microscopic identification was performed using lactophenol cotton blue (LPCB) mounts. Microscopic features such as the shape and arrangement of macroconidia, microconidia, and hyphae were documented. Identification was confirmed according to standard taxonomic keys (Gupta et al., 2023; McTaggart et al., 2025).

Data Analysis

Data were analyzed descriptively. The prevalence of dermatophytes was expressed as the percentage of positive cases among the samples examined. The distribution of species was presented in frequency tables. Associations between clinical site (nail vs. interdigital) and dermatophyte species were evaluated using the chi-square test with a significance level of $p < 0.05$ (SPSS version 26.0; IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Sample Characteristics

A total of 100 students participated in this study, consisting of 42 males (42%) and 58 females (58%), with an age range of 18–24 years (mean = 20.6 ± 1.4 years). Of the samples collected, 60 were toenail clippings and 40 were interdigital scrapings.

Direct Microscopy (KOH Examination)

100 samples, 65 (65%) showed positive findings for fungal elements, including septate hyphae and arthroconidia. The positivity rate was higher in toenail samples (68.3%) than interdigital scrapings (60%).

Culture Results

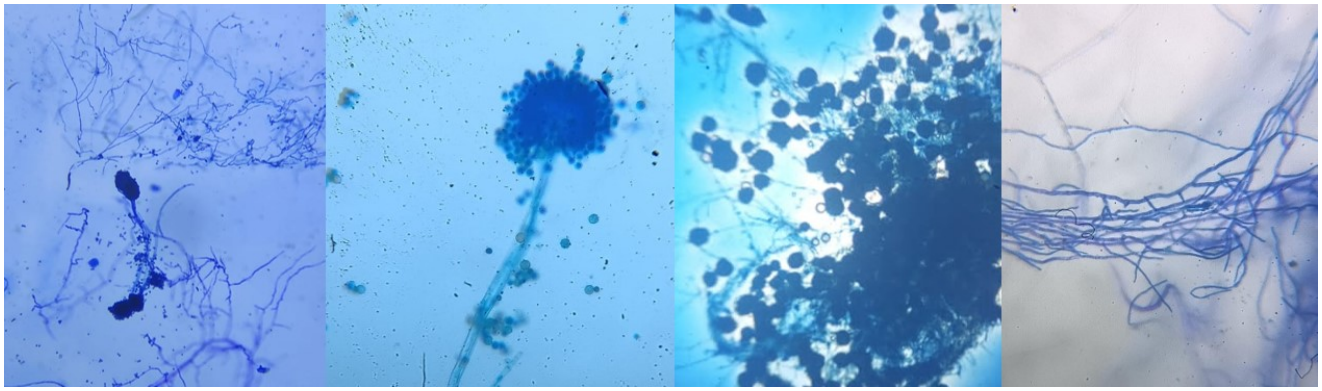
Dermatophyte growth was observed in 52 samples (52%) after incubation on Czapek Yeast Extract Agar (CYA). Contaminant molds (non-dermatophytes) were detected in 6 samples, while 42 showed no fungal growth.

Table 1. Distribution of Dermatophytes Isolated on CYA Medium.

Species Identified	Toenails (n=60)	Interdigital spaces (n=40)	Total (n=100)	Percentage (%)
Trichophyton rubrum	18	10	28	28%
Trichophyton mentagrophytes	12	6	18	18%
Trichophyton indotineae	3	2	5	5%
Epidermophyton floccosum	1	0	1	1%
Total dermatophytes	**34**	**18**	**52**	**52%**

Morphological Findings

Colonies of *T. rubrum* (Fig. 1a) appeared as white, cottony to powdery on the surface with a red-brown reverse pigmentation. *T. mentagrophytes* (Fig. 1b) showed granular colonies with a yellowish to cream color. *T. indotineae* (Fig. 1c) isolates exhibited rapid growth with flat, spreading colonies, and microscopy revealed abundant spherical to pyriform microconidia. *E. floccosum* (Fig. 1d) showed slow growth with khaki-colored colonies and characteristic club-shaped macroconidia.



a **b** **c** **d**

Figure 1. a) *T. rubrum*, b) *T. mentagrophytes*, c) *T. indotineae*, and d) *E. floccosum.*

Statistical Analysis

There was no significant difference between the prevalence of dermatophytes isolated from toenails (56.7%) and interdigital spaces (45.0%) ($\chi^2 = 1.25$, $p = 0.26$). However, *T. rubrum* was the most common species in both sites, accounting for 28% of all isolates.

Discussion

This study demonstrated that dermatophytes were isolated from 52% of clinical samples collected from toenails and interdigital spaces of university students. The prevalence observed here is consistent with previous studies reporting onychomycosis and tinea pedis as common superficial fungal infections among young adults. For example, Lee et al. (2023) found a prevalence of 51.5% for tinea pedis among South Korean students, with *T. rubrum* as the dominant etiologic agent. Similarly, Gupta, Versteeg, and Shear (2022) emphasized that dermatophytes remain the leading cause of nail and interdigital infections worldwide.

The predominance of *T. rubrum* (28%) and *T. mentagrophytes* (18%) in this study aligns with global epidemiological trends, where these two species account for the majority of dermatophytosis cases (Gupta, Venkataraman, & Hall, 2023). Interestingly, *T. indotineae* was detected in 5% of isolates, which deserves attention. First described in India, *T. indotineae* is now recognized as an emerging, terbinafine-resistant dermatophyte that has spread to Europe and North America (Chowdhary et al., 2022; McTaggart et al., 2025; Marbaniang et al., 2025). The presence of *T. indotineae* in a young, otherwise healthy population highlights the potential for wider dissemination and the need for ongoing surveillance outside high-risk clinical settings.

Culture on Czapek Yeast Extract Agar (CYA) successfully supported dermatophyte growth, with distinct morphological characteristics observed across species. Although CYA is not traditionally used for routine dermatophyte culture, the current findings support previous observations that its nutrient composition can facilitate colony development and pigment production useful for species differentiation (Ahmadi et al., 2012; Kumar, Patel, & Singh, 2025). The detection rate of 52% was comparable to reports using Sabouraud Dextrose Agar, suggesting that CYA may

serve as a viable alternative medium for academic or diagnostic purposes, particularly in resource-limited laboratories.

The higher prevalence of dermatophytes in toenails (56.7%) compared with interdigital spaces (45.0%) was not statistically significant ($p = 0.26$). Nevertheless, this trend is consistent with the view that nails act as reservoirs for dermatophytes and may serve as sources of recurrent infection for surrounding skin (Gupta et al., 2023).

The strengths of this study include its focus on a young adult population and the use of CYA medium, which has not been widely evaluated for dermatophyte isolation. However, some limitations should be noted. First, molecular confirmation of isolates was not performed, which may limit accuracy in distinguishing closely related species. Second, the study was confined to a single institution, and may not reflect the general population.

CONCLUSIONS

This study confirmed that dermatophytes are prevalent among university students, with *T. rubrum* as the dominant species, followed by *T. mentagrophytes* and the emerging *T. indotineae*. Using Czapek Yeast Extract Agar proved effective for fungal isolation and morphological characterization. These findings highlight the importance of routine surveillance in young adults and support the potential utility of CYA as an alternative medium for dermatophyte research and diagnostics. Future studies incorporating molecular methods and multicenter sampling are recommended to strengthen epidemiological insights and guide antifungal management strategies.

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