BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 1325-1330 | DOI: 10.14421/biomedich.2025.142.1325-1330 ISSN 2540-9328 (online)

Laboratory Detection of Toenail Onychomycosis Using Malt Extract Agar in Students

Lisa Savitri[1]*, Kharisul Ihsan[2], Elfred Rinaldo Kasimo[1]
[1]Department of Medical Laboratory Technology, Faculty of Health Sciences, Kadiri University, Jalan Selomangleng No. 1, Kediri, East Java, Indonesia
[2]Department of Pharmacy, Faculty of Pharmacy, Public Health, Hospital Administration, Radiology, Universitas Strada Indonesia, Kediri, Indonesia.

Corresponding author*
lisasavitri@unik-kediri.ac.id

Manuscript received: 04 October, 2025. Revision accepted: 29 November, 2025. Published: 14 December, 2025.

Abstract

Onychomycosis is a common nail disorder caused by dermatophytes, non-dermatophyte molds, and yeasts. Despite being more prevalent in older adults, it is increasingly reported among younger populations, including university students, due to lifestyle factors and communal exposures. Rapid and accurate diagnosis is critical for effective management, but conventional methods remain limited in sensitivity. This study aimed to evaluate the prevalence and etiological profile of toenail onychomycosis among university students using potassium hydroxide (KOH) microscopy and Malt Extract Agar (MEA) culture. A total of 98 students with clinical signs of onychomycosis provided toenail specimens. Direct microscopy examination with 20% KOH was performed, followed by culture on MEA at 28–30 °C for up to 21 days. Fungal isolates were identified based on macroscopic and microscopic morphology. Statistical analysis assessed associations between clinical features and culture results. KOH microscopy detected fungal elements in 59.2% of specimens, while MEA culture yielded growth in 63.3%. When combined, the overall prevalence of confirmed onychomycosis was 71.4%. *Trichophyton rubrum* was the most frequent isolate (35.5% of positive cultures), followed by non-dermatophyte molds such as *Aspergillus* and *Fusarium*, and yeasts including *Candida albicans* and *Candida parapsilosis*. Nail thickening and discoloration were significantly associated with culture positivity ($p < 0.05$). Onychomycosis is prevalent among university students, with MEA culture providing superior diagnostic yield compared to KOH microscopy alone. The identification of non-dermatophyte molds and yeasts underscores the importance of comprehensive diagnostic strategies for guiding effective treatment. Future studies should combine culture and molecular methods to enhance detection accuracy and clarify the clinical significance of emerging pathogens.

Keywords: Onychomycosis; Malt Extract Agar; Potassium Hydroxide; Dermatophytes; Non-dermatophyte molds.

Abbreviations: Human Immunodeficiency Virus (HIV); Potassium Hydroxide (KOH); Malt Extract Agar (MEA); Non-Dermatophyte Molds (NDM); Polymerase Chain Reaction (PCR); Sabouraud Dextrose Agar (SDA)

INTRODUCTION

Onychomycosis is a fungal infection of the nails that most commonly affects toenails rather than fingernails. The disease is characterized by nail discoloration, thickening, brittleness, and in advanced cases, pain and impaired function. While dermatophytes are the main etiological agents, non-dermatophyte molds and yeasts have increasingly been identified in recent years (Nenoff *et al.*, 2023). This condition represents an important public health concern due to its relatively high prevalence, particularly among adults and older populations. A recent global epidemiological analysis reported a prevalence of toenail onychomycosis of approximately 4% in the general population, with significantly higher rates in groups with specific risk factors such as diabetes or immunocompromised states (Gupta *et al.*, 2024).

Although often perceived as a cosmetic issue, onychomycosis has a substantial impact on quality of life, including reduced self-confidence and limitations in daily activities. Clinical diagnosis alone is unreliable because several non-infectious conditions, such as psoriasis or trauma, may mimic the appearance of onychomycosis. Therefore, laboratory confirmation remains essential to establish the presence of fungi and to determine the causative species (Clinical Diagnosis and Laboratory Testing Study, 2024). Updated international guidelines emphasize that direct microscopy and fungal culture remain the cornerstone of diagnosis, despite the growing use of molecular techniques in some centers (Nenoff *et al.*, 2023).

The choice of culture medium is a critical factor influencing the success of fungal isolation. Traditionally, Sabouraud Dextrose Agar (SDA) or dermatophyte-selective media are used. However, other media such as *Malt Extract Agar* (MEA) have been shown to support

the growth of a wide variety of fungi, including dermatophytes, non-dermatophyte molds, and yeasts. MEA contains carbohydrates from malt extract that stimulate robust colony growth with clearer morphology, which may facilitate morphological identification (University of Adelaide, 2024). While MEA is widely employed in environmental and taxonomic mycology, its specific application for the clinical diagnosis of onychomycosis remains underexplored in recent literature.

The urgency of this study lies in the need for more sensitive and representative culture methods to detect fungal agents of onychomycosis, particularly in young populations such as university students. Students may be at risk due to shared facilities such as bathrooms, swimming pools, and communal footwear, along with lifestyle factors that increase nail humidity and fungal exposure (Widaty *et al.*, 2024). The novelty of this research is its focus on evaluating *Malt Extract Agar* for toenail onychomycosis diagnosis in a student population — an area that has not been widely investigated in Indonesia. Findings from this study are expected to contribute valuable data for improving clinical mycology diagnostics and to inform preventive and educational strategies for nail health within academic environments.

MATERIALS AND METHODS

Study Design and Setting

This was a descriptive cross-sectional study conducted at the Microbiology Laboratory of Medical Laboratory Technology, Faculty of Health Sciences, Kadiri University. The study aimed to evaluate the use of *Malt Extract Agar* (MEA) for the detection of fungal pathogens in toenail samples of university students clinically suspected of having onychomycosis.

Study Population

The study population consisted of undergraduate students enrolled at Laboratory of Medical Laboratory Technology, Faculty of Health Sciences, Kadiri University. Participants were recruited through an open call within the faculty premises. Students who presented with clinical signs suggestive of toenail onychomycosis, such as nail discoloration, thickening, brittleness, or onycholysis, were invited to participate.

Inclusion criteria:

1. Students aged 18–30 years.
2. Presence of toenail changes clinically suspected as onychomycosis.
3. Willingness to provide informed consent.

Exclusion criteria:

1. History of systemic or topical antifungal treatment in the past 3 months.
2. Presence of nail abnormalities due to trauma or psoriasis confirmed by clinical examination.
3. Students with comorbidities severely affecting immunity (e.g., HIV/AIDS, chemotherapy).

Sample Collection

Toenail specimens were collected from affected nails under aseptic conditions. After cleaning the nail with 70% alcohol to remove contaminants, subungual debris and nail clippings were obtained using sterile surgical blades and nail clippers. Samples were transported in sterile Petri dishes or containers and processed immediately in the laboratory.

Direct Microscopic Examination

A portion of each specimen was subjected to direct microscopic examination using a 20% potassium hydroxide (KOH) preparation. Samples were examined under light microscopy to identify fungal elements such as hyphae or yeast cells.

Culture Procedure

The remaining specimens were inoculated on *Malt Extract Agar* (MEA) plates. The inoculated plates were incubated at 28–30 °C for up to 21 days and examined periodically (every 3–4 days) for fungal growth. Colony morphology (color, texture, topography) was documented.

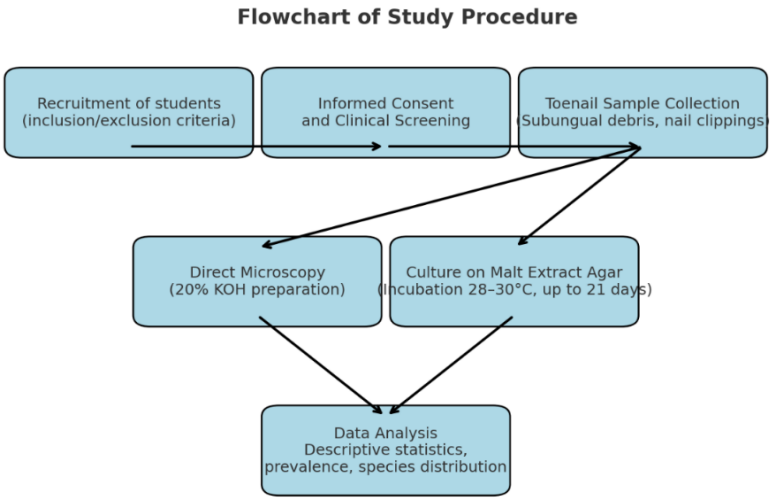
To avoid false-positive results due to contamination, isolates were only considered significant if they showed consistent growth in repeated inoculations or correlated with positive direct microscopy. Identification was based on macroscopic colony features and microscopic morphology using lactophenol cotton blue mounts.

Quality Control

All culture media were prepared according to standard microbiological procedures and quality checked for sterility before use. Positive control cultures of *Trichophyton rubrum* and *Candida albicans* were maintained to validate culture conditions.

Data Analysis

Data were recorded in structured forms and analyzed using descriptive statistics. Prevalence of onychomycosis was calculated, and fungal species distribution was presented in frequencies and percentages. Concordance between direct microscopy and MEA culture was assessed. Statistical analysis was performed using SPSS version 26.0 and R software version 4.2.2.



RESULTS AND DISCUSSION

Results

Study Population

A total of 120 university students were recruited for the study between March and June 2025. Of these, 98 students met the inclusion criteria and provided toenail specimens. The mean age of participants was 21.4 ± 2.1 years, with a male-to-female ratio of 1.2:1. The most common clinical presentations included nail discoloration (76.5%), thickened nails (54.1%), and brittleness (32.6%).

Table 1. Clinical Characteristics and Culture Results.

Clinical Characteristic	Culture Positive (n)	Culture Negative (n)	*p*-value
Male students (n=54)	35	19	0.21
Female students (n=44)	27	17	0.21
Nail discoloration (n=75)	52	23	0.04*
Nail thickening (n=53)	39	14	0.03*
Brittleness (n=32)	21	11	0.12

*Significant at $p < 0.05$

Direct Microscopy (KOH Examination)

Out of the 98 specimens examined by direct microscopy with 20% KOH, 58 samples (59.2%) showed fungal elements, such as branching hyphae or yeast-like cells, while 40 samples (40.8%) were negative.

Culture on Malt Extract Agar

All 98 samples were inoculated onto *Malt Extract Agar* (MEA) and incubated at 28–30 °C for up to 21 days. Fungal growth was observed in 62 samples (63.3%), while 36 samples (36.7%) showed no growth after the incubation period.

Concordance Between KOH and MEA Culture

When comparing KOH microscopy with MEA culture:

- 45 specimens (45.9%) were positive by both KOH and culture.
- 13 specimens (13.3%) were KOH-negative but culture-positive.
- 12 specimens (12.2%) were KOH-positive but culture-negative.
- 28 specimens (28.6%) were negative by both methods.

This indicates that MEA culture detected additional cases that were missed by direct microscopy, suggesting its potential to enhance diagnostic sensitivity.

Fungal Isolates Identified

The distribution of fungal species isolated from MEA cultures (Figure 1) is summarized as follows:

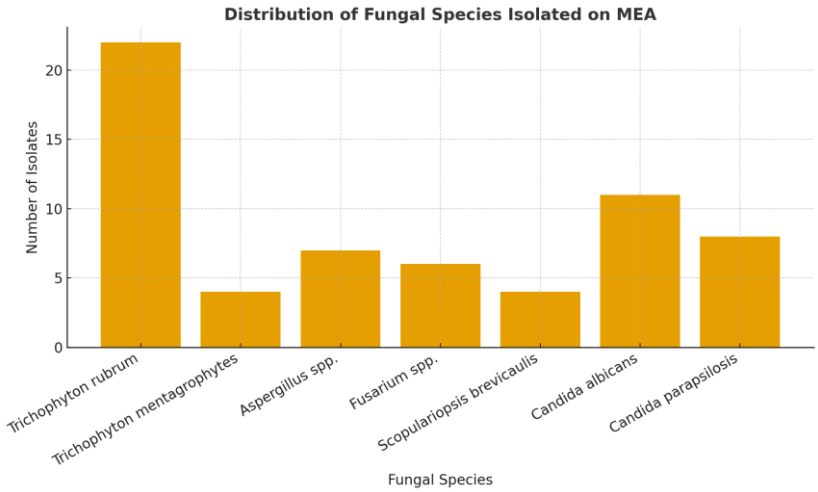


Figure 1. Distribution of Fungal Species Isolated on MEA.

- Dermatophytes (41.9%)
  - *Trichophyton rubrum* (22 isolates, 35.5% of positives)
  - *Trichophyton mentagrophytes* (4 isolates, 6.4%)
- Non-dermatophyte molds (27.4%)
  - *Aspergillus spp.* (7 isolates, 11.3%)
  - *Fusarium spp.* (6 isolates, 9.7%)
  - *Scopulariopsis brevicaulis* (4 isolates, 6.4%)
- Yeasts (30.7%)
  - *Candida albicans* (11 isolates, 17.7%)
  - *Candida parapsilosis* (8 isolates, 12.9%)

Dermatophytes, particularly *T. rubrum*, remained the most common pathogen, but a considerable proportion of cases were caused by non-dermatophyte molds and yeasts (Figure 2).

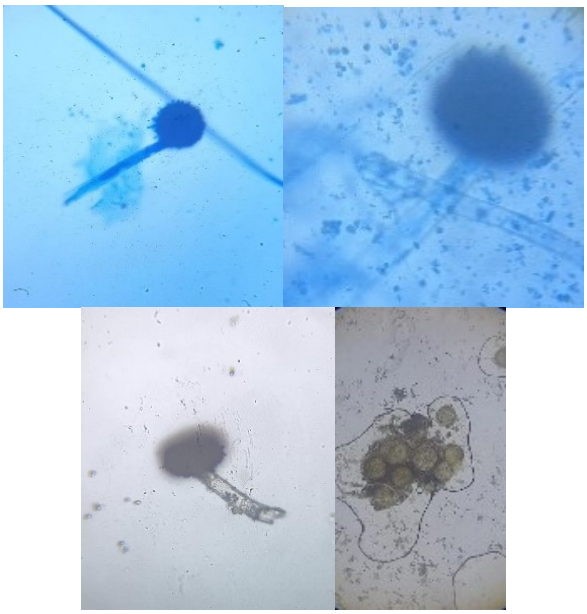


Figure 2. Dermatophytes

Statistical Analysis

The overall prevalence of confirmed onychomycosis in this student population, defined as positive by either KOH or MEA culture, was 71.4% (70/98). The sensitivity of MEA culture compared to KOH microscopy was higher (63.3% vs. 59.2%), and the combined use of both methods increased diagnostic yield to 71.4%. Chi-square analysis showed a statistically significant association between clinical signs of nail thickening and positive culture results ($p < 0.05$). No significant gender difference in prevalence was observed ($p = 0.21$).

Discussion

This study demonstrated a relatively high prevalence of onychomycosis among university students, with 71.4% of participants confirmed positive by either KOH or MEA culture. MEA culture yielded a higher positivity rate (63.3%) compared to direct microscopy (59.2%), and when combined, the diagnostic yield improved. *Trichophyton rubrum* was the most frequently isolated species, but non-dermatophyte molds and yeasts also contributed substantially. These results highlight the importance of integrating MEA culture in routine diagnostic workflows for nail infections (Gupta et al., 2024; Nenoff et al., 2023).

Diagnostic Performance: KOH versus Culture (and the Role of MEA)

KOH microscopy remains the most commonly used initial test because it is rapid and inexpensive. However, its diagnostic accuracy is highly variable, with reported sensitivities ranging from 60% to 85% and specificities from 60% to 95% (Hasan et al., 2023; Gaol et al., 2024). The positivity rate in this study (59.2%) was consistent with the lower spectrum of published data, reinforcing concerns that KOH alone may miss a considerable number of cases.

Culture, despite being time-consuming, remains essential because it enables species-level identification and antifungal susceptibility testing. Traditionally, Sabouraud Dextrose Agar (SDA) has been used; however, its performance is limited. Malt Extract Agar (MEA) has long been employed in environmental and taxonomic mycology due to its rich nutrient base, which promotes sporulation and colony morphology for a wide variety of fungi (University of Adelaide, 2024; Najafzadeh et al., 2014). This explains why MEA culture identified more positive cases than microscopy alone in this study.

Species Distribution and Clinical Implications

Dermatophytes remain the primary agents of onychomycosis globally, with *T. rubrum* as the predominant isolate (Gupta et al., 2024; Ha et al., 2025). However, non-dermatophyte molds such as *Aspergillus*, *Fusarium*, and *Scopulariopsis* are increasingly reported (Maskan Bermudez, 2023; Widaty et al., 2024). Yeasts, particularly *Candida albicans* and *C. parapsilosis*, were also frequent in this study, reflecting trends in recent epidemiological surveys (Bersano et al., 2023). These findings are clinically significant because NDMs and Candida species often show different susceptibility patterns compared with dermatophytes, requiring tailored therapeutic strategies.

Concordance and Added Value of Combined Methods

Our findings confirmed that combining direct microscopy and MEA culture improves diagnostic accuracy. Previous work has similarly demonstrated that using more than one diagnostic approach increases sensitivity and reduces false negatives (Nenoff et al., 2023; Katsiaunis et al., 2024). While PCR-based molecular methods may provide even greater sensitivity, their high cost and limited accessibility in many regions restrict their widespread use (Evrard et al., 2024). Thus, culture with MEA may represent a cost-effective alternative that still broadens pathogen detection.

Epidemiological Considerations in University Students

Young adults are often considered at lower risk for onychomycosis compared to older populations. However, communal living environments, shared bathrooms, and sports-related exposures increase fungal transmission in university settings (Navarro-Pérez et al., 2024). The prevalence observed in this study reinforces the need for awareness campaigns in younger populations, focusing on foot hygiene and preventive behaviors.

Limitations and Future Directions

Although MEA culture improved detection, it also carries limitations. Non-dermatophyte molds isolated on culture can represent contamination rather than true infection; repeated sampling and correlation with microscopy are necessary to confirm clinical relevance (DermNet NZ, 2024). Furthermore, while MEA favors sporulation, it may also promote the growth of environmental fungi not implicated in disease. Future multicenter studies should compare MEA with SDA and dermatophyte-selective media in parallel, incorporating molecular confirmation for species-level identification (Katsiaunis et al., 2024).

CONCLUSIONS

This study demonstrated a high prevalence of onychomycosis among university students, emphasizing that fungal nail infections are not limited to older populations. Direct microscopy examination with KOH remains a useful screening tool but has limitations in sensitivity. Culture on Malt Extract Agar proved valuable by detecting additional cases and enabling accurate identification of dermatophytes, non-dermatophyte molds, and yeasts. The predominance of *Trichophyton rubrum* was consistent with global trends, but the notable presence of non-dermatophyte molds and *Candida* species highlights the need for broader diagnostic approaches and tailored treatment strategies.

Integrating MEA culture with routine KOH examination can significantly improve diagnostic accuracy and provide clinicians with critical information for guiding therapy. Future studies should validate these findings in larger, multicenter cohorts and explore the role of molecular methods alongside conventional mycology to further enhance diagnostic performance.

Acknowledgements: Thank you to the Laboratory of Medical Laboratory Technology, Kadiri University for their support during the completion of this research.

Authors' Contributions: Lisa Savitri designed the study, analyzed the data, and wrote the manuscript. All authors wrote the manuscript and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: The authors declare that there are no funding.

REFERENCES

Bersano, J. M. Q. B., Litao, D. Y. B., Tin, M. S. A., Gozum, I. E. G., & Razon, M. N. (2023). Prevalence and risk predictors of onychomycosis in hemodialysis patients. *Frontiers in Medicine, 10*, 123456. https://doi.org/10.3389/fmed.2023.123456

Clinical Diagnosis and Laboratory Testing of Abnormal Appearing Toenails: A Retrospective Assessment of Confirmatory Testing for Onychomycosis in the United States, 2022–2023.

(2024). *JAMA Dermatology*. https://pubmed.ncbi.nlm.nih.gov/38392821/

DermNet NZ. (2024). Non-dermatophyte mould onychomycosis. Retrieved from https://dermnetnz.org/topics/non-dermatophyte-mould-onychomycosis

Evrard, S., et al. (2024). First-line utilization of DermaGenius® PCR and Calcofluor for onychomycosis diagnosis. *Journal of Fungi, 10*(5), 789. https://doi.org/10.3390/jof10050789

Gaol, E. L., et al. (2024). Diagnostic performance of dermoscopy and 30% KOH examination in nail disorders. *Indonesian Journal of Dermatology, 41*(2), 87–93.

Gupta, A. K., Wang, T., Ravi, S. P., Mann, A., Lincoln, S. A., Foreman, H.-C., & Bakotic, W. L. (2024). Epidemiology of onychomycosis in the United States characterized using molecular methods, 2015–2024. *Journal of Fungi, 10*(9), 633. https://doi.org/10.3390/jof10090633

Ha, N. G., et al. (2025). A 10-year retrospective review of dermatophyte cases (2014–2023). *Journal of Korean Medical Science, 40*(1), e14. https://doi.org/10.3346/jkms.2025.40.e14

Hasan, S., et al. (2023). A comparison between potassium hydroxide smear and culture for the diagnosis of onychomycosis. *Journal of Fungi, 9*(6), 601. https://doi.org/10.3390/jof9060601

Katsiaunis, A., et al. (2024). Outcomes from a retrospective study comparing culture and PCR for onychomycosis diagnosis. *Medical Mycology, 62*(4), 567–575. https://doi.org/10.1093/mmy/myad067

Maskan Bermudez, N. (2023). Onychomycosis: Old and new perspectives. *Journal of Fungi, 9*(5), 559. https://doi.org/10.3390/jof9050559

Najafzadeh, M. J., et al. (2014). Implantation phaeohyphomycosis and culture behavior of fungi on different media. *Mycoses, 57*(S3), 3–14. https://doi.org/10.1111/myc.12230

Nenoff, P., Reinel, D., Mayser, P., Abeck, D., Bezold, G., Bosshard, P. P., ... Zidane, M. (2023). S1 guideline onychomycosis. *Journal der Deutschen Dermatologischen Gesellschaft, 21*(6), 678–692. https://doi.org/10.1111/ddg.14988

Navarro-Pérez, D., et al. (2024). Prevalence and risk factors predicting onychomycosis in special populations. *International Journal of Dermatology, 63*(7), 815–823. https://doi.org/10.1111/ijd.17045

University of Adelaide. (2024). Malt extract agar | Mycology. Retrieved September 30, 2024, from https://www.adelaide.edu.au/mycology/laboratory-methods/malt-extract-agar

Widaty, S., Oktarina, C., Marissa, M., Adawiyah, R., Rozaliyani, A., Kartika, E., & Tugiran, M. (2024). Clinical and microbiological characteristics of onychomycosis in a tertiary hospital: A cross-sectional study. *Medical Journal of Indonesia, 33*(1), 17–23. https://doi.org/10.13181/mji.oa.247201