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Mycological Profile of Onychomycosis: A Four-Year Retrospective Analysis from a Tertiary Care Center in South India

Arthi Elumalai[1*], Ashitha Darique[2]

[1]Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry, India; [2]MBBS Student, Pondicherry Institute of Medical Sciences, Puducherry, India

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***Correspondence**
Email: doc_aarthi@ymail.com

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ABSTRACT

Introduction: Onychomycosis (OM) is the most common nail disorder, accounting for up to 50% of nail dystrophies. The global prevalence of OM is 5.5%, and India has a reported prevalence ranging from 0.5% to 5% across different regions. The most common agents of OM are dermatophytes, particularly *Trichophyton rubrum* and *Trichophyton interdigitale*. However, Indian literature shows considerable variability in the clinical presentations and the etiological spectrum. This study was conducted to determine the clinical presentations and mycological profile of OM in a tertiary care setting in South India. **Methods:** This retrospective descriptive study, conducted over a 4-year period, reviewed records of patients attending the dermatology clinic with a clinical suspicion of OM from January 2020 to December 2023. Nail samples were processed using standard microscopic and culture methods. **Results:** Of the 172 patients with clinically suspected OM included in the study, 78 (45.3%) were laboratory-confirmed by potassium hydroxide (KOH) microscopy and/or fungal culture. Females predominated, with a male-to-female ratio of 1:1.8. Fingernails were the most commonly affected site, with fingernail involvement observed in 55.8% of the clinically suspected cases. Distal lateral subungual onychomycosis (DLSO) was the predominant clinical presentation, accounting for 55.8% of the clinically suspected cases. Among the fungal isolates, *Candida* spp. were the most frequently isolated (54.7% of the 64 culture-positive isolates), followed by *Fusarium* spp. (25.0%). Dermatophytes were isolated in only 3.1% of the culture-positive isolates (n = 2, both identified as *T. rubrum*). **Conclusion:** This study demonstrates predominance of non-dermatophyte molds (NDMs) and *Candida* spp. over dermatophytes among culture-positive isolates in our specific tertiary care setting, which contrasts with several earlier Indian studies that reported dermatophyte predominance. These findings suggest that NDMs and *Candida* spp. may be important fungal isolates in OM in similar settings, although their pathogenic significance, particularly for NDMs and yeasts, requires careful clinical and laboratory correlation.

INTRODUCTION

Antibiotic resistance remains a global health threat, with Onychomycosis (OM), a fungal infection of the nail unit, is the most common cause of dystrophic nails, accounting for up to 50% of all such cases [1, 2]. OM commonly presents with nail deformities such as nail-plate thickening, discoloration, or onycholysis [3, 4]. The global prevalence of OM is 5.5%, and the reported prevalence in India ranges from 0.5% to 5% across different regions [5, 6]. The prevalence of OM is increasing due to factors such as longer life expectancy, occlusive footwear, and prolonged exposure to moisture [7]. Other predisposing factors include hyperhidrosis, sports-related repetitive nail trauma, and coexistent cutaneous fungal infections [8, 9].

The traditional causative agents of OM are dermatophytes, particularly *Trichophyton rubrum* and *T. interdigitale*, which are responsible for approximately 90% of toenail and 75% of fingernail infections in Western literature [10, 11]. In the Indian context, several studies have historically demonstrated dermatophyte predominance. Kaur et al. reported dermatophytes as the most common etiological agents (48.89%) among 180 culture-positive OM cases in New Delhi, with *T. mentagrophytes* being the predominant isolate [12]. Similarly, Yadav et al. found that

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dermatophytes dominated the etiological spectrum in a cohort of 100 KOH- and culture-proven toenail OM cases in New Delhi, with *T. interdigitale* (61%) and *T. rubrum* (34%) as the principal species [13]. More recently, Chetana *et al*. reported dermatophyte predominance (78.6%) among 168 culture-positive OM cases from South India (2015–2017), with *T. rubrum* accounting for 45.0% of isolates [14].

However, recent studies indicate a shift toward non-dermatophyte molds (NDMs) as major isolates associated with OM in certain populations and settings, with reported prevalence rates ranging from 0.8% to 65.8% in different populations worldwide [15]. Agrawal *et al*. reported a similar shift away from dermatophytes among diabetic patients in the Indian subcontinent, in which NDMs accounted for 47.6% and *Candida* spp. for 30.15% of OM cases [16]. Similarly, Borgohain et al. reported that 52% of culture-positive cases in agricultural workers in Assam were associated with NDMs, especially dematiaceous fungi [17]. Since these studies were conducted in specific patient populations, the present study was undertaken to determine the clinical profile and prevailing mycological trends of OM in our tertiary care setting.

MATERIAL AND METHODS

Study design and setting. This retrospective descriptive study was conducted from January 2020 to December 2023 at the microbiology laboratory of a tertiary care hospital in Puducherry, India.

Inclusion criteria. The study population included all patients attending the dermatology clinic with a clinical suspicion of OM, as indicated by the presence of one or more of the following nail changes:
a) White or yellow discoloration
b) Nail plate thickening
c) Accumulation of nail debris distally
d) Onycholysis and/or chronic paronychia.

Exclusion criteria. These criteria included patients who had received antifungal therapy within the preceding four weeks as documented in the medical records.

Laboratory processing and culture. For laboratory confirmation, nail samples were subjected to direct microscopy using a 20% potassium hydroxide (KOH) preparation and culture. Approximately two to three nail fragments were cultured on two types of media: Sabouraud dextrose agar (SDA) and SDA supplemented with 0.5 g/L cycloheximide. SDA without cycloheximide was incubated at 25°C to enhance recovery of dermatophytes, while SDA with cycloheximide was incubated at 37°C to enhance the recovery of yeasts and NDMs. Both media were incubated for up to four weeks and were examined weekly for growth. The rationale for the use of cycloheximide-containing medium should be stated accurately, because cycloheximide suppresses many environmental fungi and should not be described as enhancing recovery of NDMs.

To minimize contamination, all culture processing was performed under aseptic conditions in a biosafety cabinet. Sterile uninoculated SDA plates were included as negative controls with each batch of cultures and were incubated under identical conditions. Plates showing growth of multiple different colony morphotypes or bacterial contamination were excluded from analysis.

Fungal identification. Fungal isolates were identified using conventional morphological methods, including a presumptive germ tube test, chromogenic agar, and carbohydrate fermentation and assimilation tests for yeast identification, as well as slide culture and lactophenol cotton blue preparations for mold identification. It is acknowledged that conventional morphological methods may not allow definitive species-level identification within certain species complexes (*e.g*., the *Fusarium solani* species complex), and molecular confirmation (*e.g*., ITS sequencing) was not available in this study.

Diagnostic criteria and case definition. When NDM growth was detected, predefined modified laboratory criteria were applied to distinguish potentially significant isolates from contaminants and saprophytes. These criteria were based on previously published approaches to NDM diagnosis [18, 19]. The criteria used in this study were:
1) Presence of fungal elements in microscopy and growth of > 3 colonies of the same mold in at least two consecutive samples on any culture medium; or
2) Growth of the same NDM in at least two consecutive repeat samples, even if the microscopy was negative.

Cases with a KOH microscopy-positive or culture-positive result were considered laboratory-confirmed cases of OM. For NDM cases, the diagnostic criteria described above were applied to distinguish true pathogens from contaminants.

Data collection. Relevant clinical and laboratory data were retrieved for eligible patients from the hospital information system, including demographic data, involvement of fingernails or toenails, nail morphology, and underlying comorbidities (specifically diabetes mellitus and hypertension). As this was a retrospective study, all eligible cases were included (complete case ascertainment) and recorded in the microbiology laboratory database during the study period.

Statistical analysis. Data were recorded in Microsoft Excel. Qualitative variables were described as frequencies and percentages, and quantitative variables as medians with interquartile ranges. Statistical comparisons were performed using the chi-square test in IBM SPSS Statistics version 24 (SPSS Inc., Chicago, IL, USA). *P*-values < 0.05 were regarded as statistically significant.

RESULTS

During the 4-year study period (2020–2023), 172 patients with clinically suspected OM were included in the study. Of these patients, 78 were laboratory-confirmed cases of OM (45.3%).

Age and sex distribution. The predominant age group was 40–49 years, representing 28.5% of the study population (Figure 1). Females comprised a higher proportion (111 [64.5%]) compared with males (61 [35.5%]), with a male: female ratio of 1:1.8. The median age of the study participants was 43.4 years (IQR: 31.6–54.2 years).

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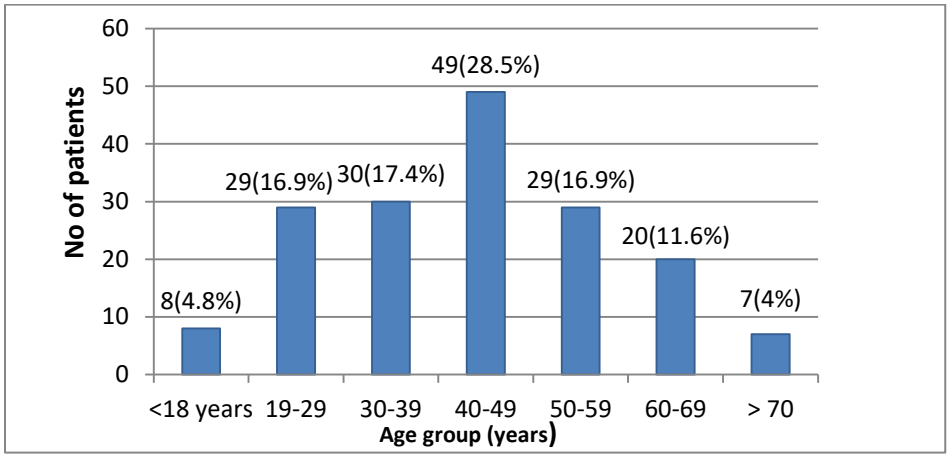


Fig. 1. Age distribution of the study population (N = 172). The bar chart illustrates the frequency and percentage of patients across distinct age groups, highlighting the highest frequency in the 40–49 year cohort.

The chi-square result (χ^2 = 45.96, df = 2, P < 0.0001) should be justified or reconsidered, as comparison with a uniform distribution is not clinically meaningful unless an equal distribution is the stated null hypothesis.

Fingernail involvement was the most frequently involved site among clinically suspected OM cases (55.8%, n = 96), followed by toenail involvement (30.2%). Involvement of both fingernails and toenails was less common (14.0%) (Table 1).

Clinical presentation of OM. Distal lateral subungual onychomycosis (DLSO) was the most common clinical subtype, observed in 96 cases (55.8%). Other presentations included total nail dystrophy in 25 cases (14.5%), onycholysis in 17 cases (9.9%), and chronic paronychia in 34 cases (19.8%) (Figure 2).

Table 1. Distribution of infection sites in patients with OM

Site of infection	Number	% (n=172)
Fingernail	96	55.8
Toenail	52	30.2
Both fingernail and toenail	24	14

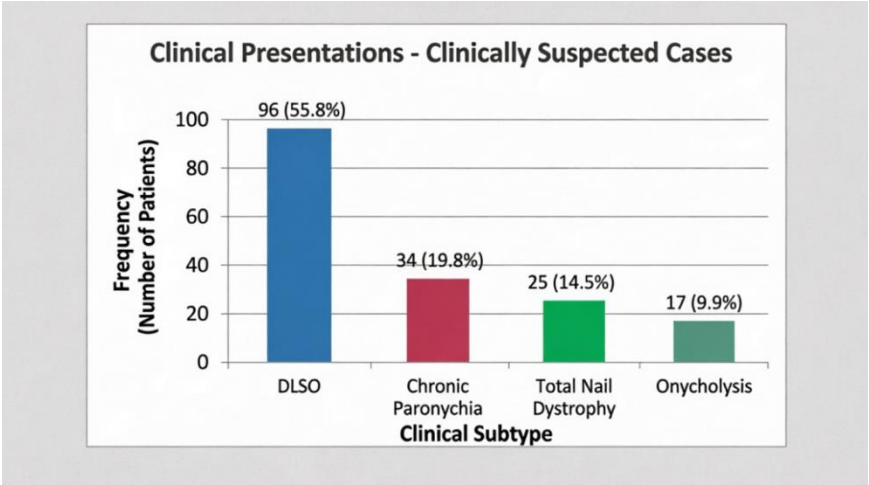


Fig. 2. Distribution of clinical presentations among the study population (N = 172). The bar chart illustrates the frequency and percentage of patients across four distinct clinical subtypes of onychomycosis. DLSO was the most common presentation, followed by chronic paronychia, total nail dystrophy, and onycholysis.

KOH and culture positivity. Among the 172 clinically diagnosed cases of OM, 78 (45.3%) were laboratory-confirmed by either KOH microscopy or culture. Of these 78 confirmed cases, 31 (39.7%) were positive by both methods, 33 (42.3%) were culture-positive only, and 14 (17.9%) were KOH-positive only (Figure 3). Among all clinically suspected cases (n = 172), KOH microscopy detected fungal elements in 45 cases (26.2%), while culture yielded growth in 64 cases (37.2%). Among laboratory-confirmed cases (n = 78), KOH positivity was 57.7% (45/78) and culture positivity was 82.1% (64/78).

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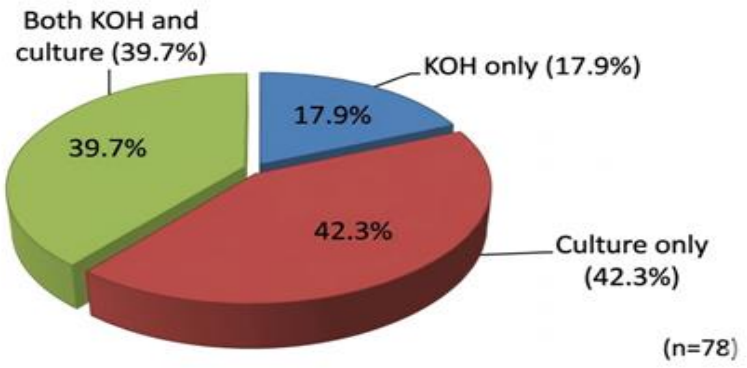


Fig. 3. Distribution of microscopy or culture positivity among the laboratory-confirmed cases of onychomycosis.

Mycological profile of agents causing OM. *Candida* spp. were the most frequently isolated organisms, accounting for 54.7% of the 64 culture-positive isolates, and among molds, *Fusarium* spp. were the most frequently isolated (25.0%). The most commonly identified species were *Candida tropicalis* and the *Fusarium solani* species complex (Table 2).

Table 2. Distribution of etiological agents isolated from OM cases (n = 64)

Isolate	Number	%
Candida	35	54.7
Candida spp.	9	14.1
Candida albicans	12	18.8
Candida tropicalis	11	17.2
Candida lusitaniae	1	1.6
C. parapsilosis	1	1.6
C. guilliermondii	1	1.6
Trichosporon	4	6.3
Trichosporon spp.	1	1.6
Trichosporon ovoides	1	1.6
Trichosporon asahii	2	3.1
Galactomyces candidus	2	3.1
Trichophyton rubrum	2	3.1
Non-dermatophyte molds (NDMs)	21	32.8
Fusarium	16	25.0
Fusarium solani	13	20.3
Fusarium dimerum	1	1.6
Fusarium oxysporum	2	3.1
***Cladosporium* spp.**	3	4.7
Aspergillus flavus	1	1.6
Cylindrocarpon lichenicola	1	1.6

Note: The identification of *Fusarium* isolates was performed using conventional morphological methods (slide culture and lactophenol cotton blue preparations). The designation "*Fusarium solani* species complex" is used to acknowledge that definitive species-level identification within this complex requires molecular methods (*e.g.*, ITS or TEF1-α sequencing), which were not available in this study. Similarly, the identification of *Galactomyces candidus* (n = 2) was based on conventional morphological and biochemical methods; molecular confirmation would be desirable to validate this uncommon isolate, and these findings should be interpreted with caution.

Analysis of risk factors showed that *Candida*-associated OM was significantly associated with female sex, age < 45 years, and fingernail involvement. However, NDM-associated OM caused by *Fusarium* spp., *Cladosporium* spp., and *Cylindrocarpon* spp. did not show significant associations with these demographic or clinical variables (Table 3). OM caused by other agents was not analyzed for risk factors due to small sample sizes.

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Table 3. Distribution of risk factors among culture-confirmed cases of OM

Risk factors	*Candida*-associated OM (n=35)	NDM-associated OM (n=21)	χ^2	*P*-value
Age < 45 years	31 (88.6%)	14 (66.7%)	4.91	0.03*
Female	31 (88.6%)	13 (61.9%)	5.54	0.02*
Fingernail involvement	32 (91.4%)	13 (61.9%)	7.22	0.01*

* $P < 0.05$ was considered statistically significant.

DISCUSSION

The epidemiological patterns and etiological agents of OM are evolving [19, 20]. Our findings provide important insights into the demographic characteristics of individuals presenting with OM. The predominant age group was 40–49 years, representing 28.5% of the study population. This is consistent with existing literature, which suggests that OM becomes more prevalent with advancing age. The age-related increase in incidence may be attributed to factors such as repeated nail microtrauma, cumulative occupational exposure, impaired peripheral circulation, venous insufficiency, immunosenescence, and prolonged exposure to environmental risk factors such as moisture or occlusive footwear, all of which are more common in older populations [17, 12].

Sex differences were also evident in our study, with females comprising a higher proportion (64.5%) of the affected individuals. This sex disparity has been reported in other studies and may be related to multiple factors. For example, female patients may be more likely to engage in activities that increase the risk of OM, such as frequent use of nail polish, regular manicures, or exposure to communal bathing facilities. Additionally, hormonal differences have been suggested to influence susceptibility to OM, as estrogen may modulate local immune responses and the tissue microenvironment, possibly facilitating fungal colonization [19, 21].

In the present study, fingernail OM was more common than toenail involvement. Kaur *et al.* demonstrated similar findings in a study conducted in New Delhi, where 65% of the study population had fingernail involvement, which was predominantly seen in females, and toenail involvement was predominantly seen in males [12]. This difference may be related to occupational activities involving prolonged moisture exposure among female patients and to repeated foot trauma and occlusive footwear use among males engaged in outdoor activities.

DLSO was the most common clinical subtype of OM, accounting for 55.8% of clinically suspected cases, which is consistent with other similar studies [22]. This finding was notable given the low isolation rate of dermatophytes; however, DLSO can also be caused by non-dermatophyte molds and *Candida* spp., and the clinical-mycological correlation in this cohort warrants further investigation with prospective sampling and molecular methods

The KOH microscopy positivity rate among laboratory-confirmed cases was 57.7%, whereas culture was positive in 82.1% of laboratory-confirmed cases. Among all clinically suspected cases, the culture positivity rate was 37.2%. Kaur *et al.* reported KOH and culture positivity rates of 34% and 45%, respectively [12]. This finding may be attributed to the lower sensitivity of direct microscopy, which may miss fungal elements due to inadequate sampling or preparation, whereas culture requires viable organisms for growth and, in this study, yielded a higher detection rate. KOH detects fungal elements regardless of viability but may be limited by technician experience and sample quality, whereas culture provides greater specificity for organism identification. Similar studies conducted by Chetana *et al.* reported KOH and culture positivity rates of 41.1% and 59.1%, respectively, and Kayarkatte *et al.* reported a culture positivity rate of 53.4% [14, 23]. The lower culture positivity rate compared with KOH microscopy could be attributed to factors such as improper sample collection, inadequate specimens, or the presence of non-viable fungal elements due to undisclosed prior antifungal therapy or herbal remedies. As this was a retrospective study, these methodological limitations could not be excluded. Therefore, KOH microscopy remains a simple, rapid test for the diagnosis of OM, whereas culture remains a more specific method for confirming infection and identifying the causative pathogen.

Among the etiological agents, *Candida* spp. were the most frequently isolated among culture-positive cases, accounting for 54.7% of the 64 culture-positive isolates, followed by *Fusarium* spp. (25.0%). Dermatophytes were isolated in only 3.1% of culture-positive isolates. These figures represent isolation rates among culture-positive cases, not overall population prevalence. This contrasts with several earlier Indian studies that demonstrated dermatophyte predominance. Chetana *et al*. (2019) reported dermatophytes in 78.6% of culture-positive OM cases from South India, with *T. rubrum* as the predominant species (45.0%) [14]. Kaur *et al.* (2007) found dermatophytes in 48.89% of culture-positive cases in New Delhi [12], and Yadav *et al*. (2015) confirmed dermatophyte dominance in toenail OM in the same region [13]. The present findings therefore suggest a setting-specific predominance of NDMs and *Candida* spp. rather than a universal epidemiological shift across India. A similar study conducted in India by Agrawal *et al.* demonstrated that

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47.6% of causative agents were NDMs, *Candida* spp. accounted for 30.15%, and dermatophytes for 22.2% [16]. The lower isolation rate of dermatophytes could be attributed in part to prior use of antifungal medication for other dermatophytoses. However, the predominance of NDMs and *Candida* spp. in our cohort may also be related to climatic variations, with warmer temperatures in tropical regions favoring the growth of NDMs. The predominance of *Candida*-associated OM among females and its fingernail predilection correlate with increased risk of wet-work activities and chronic exposure to moisture and chemicals such as detergents [24–26].

Although the study was retrospective, where possible, repeat samples were evaluated to differentiate colonizers from true pathogens, especially for NDMs, according to the Shemer criteria. However, the retrospective design limited the ability to obtain repeat samples in all cases, which may have affected the classification of some NDM isolates. Procedures such as histochemical staining (periodic acid–Schiff [PAS] staining) or molecular testing could not be performed to explain the low isolation rates of dermatophytes from nail samples in our study. Additionally, the identification of certain isolates (*e.g., Fusarium solani* species complex and *Galactomyces candidus*) was based solely on conventional morphological methods, and molecular confirmation was not available. These identifications should be interpreted with caution, and future prospective studies incorporating ITS sequencing or other molecular tools are warranted to validate the etiological spectrum.

In conclusion, this study indicates that *Candida* spp. and NDMs predominated over dermatophytes as causative agents of OM in our tertiary care setting. These findings should be confirmed by larger prospective multicenter studies and may inform the development of local treatment guidelines to optimize the management of OM.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

FUNDING

This research received no external funding.

AI DISCLOSURE

No artificial intelligence (AI)-assisted technologies were used in the preparation of this manuscript.

DATA AVAILABILITY

The data supporting the findings of this study were retrieved from Pondicherry Institute of Medical Sciences. Derived data are available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTION

AD: Data collection, analysis, and drafting of the initial version of the manuscript. AE: Conceived and designed the study, interpreted the data, and critically revised the manuscript. All authors reviewed and approved the final manuscript.

ETHICS STATEMENT

The study protocol was reviewed and approved by the Institute Ethics Committee in 2024 (approval no. RC/2024/04). Clinical records from 2020 to 2023 were retrospectively reviewed for this study. Patient confidentiality was maintained by removing patient identifiers and assigning unique study identification numbers to each case throughout data retrieval and analysis. The study was conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived by the Institute Ethics Committee due to the retrospective nature of the study, in accordance with institutional policy.

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