

# Onychomycosis: Dermatophytes and Yeasts to Non-dermatophytic moulds: a rising trend in Central India

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## Abstract:

**Background:** Onychomycosis is common infection in adults and accounts for 20 % of all nail diseases. It is rarely life threatening but can affect patient's quality of life by associated morbidity and cosmetic disfigurement. The current study was undertaken to know the epidemiological and mycological aspects of onychomycosis.

**Methods:** Nail samples from clinically suspected cases of onychomycosis, were subjected to KOH examination and culture on Sabouraud's Dextrose Agar.

**Results:** Infection was common in males with male to female ratio of 1.09. Young adults in age group of 31-40 years (30.58 %) were predominantly affected. Out of 291 specimen, 101 (34.71 %) were positive for fungal hyphae and spores by direct KOH preparation and 95 (32.65 %) revealed growth on culture. Onychomycosis was mostly caused by non-dermatophytic moulds (56.84 %), followed by yeasts (26.32 %) and dermatophytes (16.84 %). *Aspergillus nidulans* (14.74 %) and *A. glaucus* (7.37 %) were the most common moulds, *Trichophyton tonsurans* (6.32 %) and *T. mentagrophytes* (6.32 %) were the most common dermatophytes, while *Candida albicans* (14.74 %) was the most common yeast isolated.

**Conclusion:** Present study demonstrates a shift in agents causing onychomycoses from dermatophytes and yeasts to non-dermatophytic moulds

**Keywords:** Onychomycosis, Non-dermatophytes moulds, *Trichophyton*, *Aspergillus nidulans*.

## Introduction:

Onychomycosis is traditionally referred to non-dermatophytic infection of nail but now used as general term to denote all fungal infections of nail viz; dermatophytes, yeasts or non-dermatophytes. <sup>[1]</sup> Onychomycosis is a common condition accounting for up to 18–50 % of all nail diseases and 30 % of cutaneous fungal infections. <sup>[2]</sup> The global burden of fungal nail, skin and hair infections is about 1 billion cases, translating to nearly 300 million cases of onychomycosis globally. <sup>[3]</sup>

Various risk factors associated with this condition are reduced peripheral circulation, diabetes, nail trauma, difficulty to maintain proper nail hygiene, chronic smoking, communal bathing, etc. <sup>[4]</sup> It is not a life threatening condition but causes cosmetic problem which can have significant negative effects on patient's emotional, social, occupational functioning. Also infected nail can serve as a chronic reservoir, giving rise to repeated mycotic infection. <sup>[5]</sup>

Onychomycosis can be confused with other skin conditions like psoriasis, lichen planus, onychogryphosis, etc. <sup>[6]</sup> so, it is necessary to have a clinical suspicion and accurate laboratory diagnosis before starting treatment for appropriate results. Also, it is the most difficult to treat superficial mycosis and it is a chronic infection that is prone to relapse. <sup>[7]</sup> Therefore, it is important to identify the causative agent viz; yeast, dermatophyte or non-dermatophyte to the species level for better clinical outcome. This study was conducted to know the epidemiological and mycological aspects of onychomycosis.

## Material and methods:

This is an observational study conducted from April, 2019 to September, 2022. A total of 291 samples of patients attending Outdoor Patient Department of Dermatology, Venereology and Leprology department of our institute with suspicion of onychomycosis were included in this study. Before collecting the nail scraping, relevant history was taken. The lesion area was cleaned with 70% alcohol before sampling to remove contaminants such as bacteria. All the specimens were subjected to 10% potassium hydroxide (KOH) for a period of 24 hours and examined for the presence of fungal hyphae and spores. Two Sabouraud's Dextrose Agar (SDA) slopes with and without cycloheximide and chloramphenicol were used for culture of specimens and incubated at temperatures of 25°C and 37°C for 3 weeks for isolation of dermatophytic and non-dermatophytic moulds and yeasts. Any fungal growth was identified based on colony morphology, pigmentation, growth rate, ), (Figures d) microscopy using Lactophenol cotton blue mount (LPCB), (Figures e and f) slide culture on corn meal agar, urease test, etc. as per conventional techniques. For candida speciation, germ tube test, slide culture and hi-chrome candida chromogenic agar were used. <sup>[8]</sup>

**Results:****Table 1 - Distribution of Fungi according to KOH and culture positivity**

	Culture positive	Culture negative	Total
KOH positive	75	26	101
KOH negative	20	170	190
Total	95	196	291

**Table 2 – Fungal isolates obtained from the clinical samples**

	Fungal Isolates	No. of isolates (%)
Dermatophytes (16.84%)	<i>Trichophyton tonsurans</i>	6 (6.32)
	<i>Trichophyton mentagrophytes</i>	6 (6.32)
	<i>Trichophyton rubrum</i>	2 (2.11)
	<i>Trichophyton schoenleinii</i>	1 (1.05)
	<i>Trichophyton violaceum</i>	1 (1.05)
Moulds (56.84%)	<i>Aspergillus nidulans</i>	14 (14.74)
	<i>Aspergillus glaucus</i>	7 (7.37)
	<i>Aspergillus niger</i>	6 (6.32)
	<i>Aspergillus fumigatus</i>	2 (2.11)
	<i>Aspergillus flavus</i>	1 (1.05)
	<i>Aspergillus versicolor</i>	1 (1.05)
	<i>Fusarium spp.</i>	5 (5.26)
	<i>Mucor spp.</i>	2 (2.11)
	<i>Alternaria spp.,</i>	2 (2.11)
	<i>Hormonema dematioides,</i>	2 (2.11)
	<i>Chaetomium spp.</i>	2 (2.11)
	<i>Exophiala werneckii</i>	2 (2.11)
	<i>Rhizopus spp.</i>	1 (1.05)
	<i>Scytalidium dimidiatum</i>	1 (1.05)
	<i>Curvularia lunata</i>	1 (1.05)
	<i>Curvularia geniculata</i>	1 (1.05)
	<i>Natrassia mangiferae</i>	1 (1.05)
	<i>Chrysosporium spp.</i>	1 (1.05)
	<i>Phialemonium spp.</i>	1 (1.05)
	<i>Cladophialophora boppi</i>	1 (1.05)
Yeasts (26.32%)	<i>Candida albicans</i>	14 (14.74)
	<i>Candida tropicalis</i>	8 (8.42)
	<i>Candida glabrata</i>	3 (3.16)
Total		95 (100)

Two hundred and ninety one nail samples from clinically suspected patients were processed. Males were 152/291 (52.23 %) and females were 139/291 (47.77 %) with male to female ratio of 1.09. Maximum incidence of cases was recorded in winter season i.e. 106/291 (36.43 %) followed by summer i.e. 94/291 (32.30 %) and least in rainy season i.e. 91/291 (31.27 %). Most of the patients belonged to the age group of 31-40 years i.e. 89/291 (30.58 %). In the age group of 41-50 years, 72/291 (24.74 %) patients were seen and in that of 21-30 years, only 61/291 (20.96 %) patients were found.

Out of 291 specimen, 101 (34.71 %) were positive for fungal hyphae and spores by direct KOH preparation and 95 (32.65 %) revealed growth on culture. Twenty specimen that were negative in KOH examination revealed growth on culture (6.87%). Twenty six specimen which were positive by microscopy failed to grow in culture (8.93%). Both microscopy and culture negative specimen were 170/291 (58.42%) (Table 1)

Out of 95 culture positive specimen, 54 (56.84 %) isolates belonged to non-dermatophytic moulds, 25 (26.32 %) isolates belonged to yeasts and 16 (16.84 %) were dermatophytes. The most common non-dermatophytic mould was *Aspergillus spp.* (31/54=57.41 %) followed by *Fusarium spp.* (5/54=9.26 %). *Aspergillus nidulans* (14/31=45.16 %) and *Aspergillus glaucus* (7/31=22.58 %) were the most common *Aspergillus spp.* Other *Aspergillus* species isolated were *A.niger* (6/31), *A.fumigatus* (2/31), *A.flavus* (1/31) and *A.versicolor* (1/31). There were 2 cases each of *Mucor spp.*, *Alternaria spp.*, *Hormonema dematioides*, *Chaetomium spp.* and *Exophiala werneckii* and 1 case each of *Rhizopus spp.*, *Scytalidium dimidiatum*, *Curvularia lunata*, *Curvularia geniculata*, *Natrassia mangiferae*, *Chrysosporium tropicum*, *Phialemonium spp.* and *Cladophialophora boppi* (Table 2)

Among the yeasts, *Candida albicans* (14/25=56 %) was the most common isolate followed by *Candida tropicalis* (8/25=32 %) and *Candida glabrata* (3/25=12 %). (Table 2)

*Trichophyton tonsurans* (6/16=37.5 %) and *Trichophyton mentagrophytes* (6/16=37.5 %) were the prevalent dermatophytes. Other dermatophytes isolated were 2 cases of *T. rubrum*, 1 case each of *T. violaceum* and *T. schoenleinii*. (Table 2)

### Discussion:

Due to lack of conclusive data on onychomycosis from our region as well as other parts of India, this study was conducted to know the epidemiological and mycological aspects of onychomycosis. In our study, the infection was more common in males as compared to the females with a male: female ratio of 1.09, which was in accordance with study by Kaur et al.<sup>[9]</sup> While few studies have shown higher prevalence of this infection in females,<sup>[10, 11]</sup> the increased incidences in males, might be due to more outdoor exposure, physical activity and occupational nail trauma in males as compared to females. A higher number of patients were in the age group of 31-40 years (30.58%) in our study. Similar high prevalence in this age group was also seen in other studies.<sup>[12, 13]</sup> The younger age group of prevalence might be due to more of occupation related trauma, and cosmetic consciousness amongst the young adults as compared to elderly. However, in our study the next most common age group was 41-50 years (24.74%) which showed an increasing incidence of this infection with an advancing age. A similar result was also seen in study conducted by Veer et al.<sup>[12]</sup> this high incidence with an advancing age might be due to a decrease in immunity with age, due to repeated nail trauma, or decreased peripheral circulation, personal habits like chronic smoking and antecedent diseases like diabetes, peripheral vascular disease in elderly age groups.

Direct microscopy by KOH mount is important for clinical diagnosis while culture is required to identify the pathogenic fungus. The results of both may vary as direct microscopy is relatively easy but subjective, while culture needs technical expertise.<sup>[14]</sup> In our study, 41.58 % cases were positive by KOH and/or culture. In studies which were conducted by Lone et al,<sup>[15]</sup> Aghamirian et al,<sup>[10]</sup> Kaur et al,<sup>[9]</sup> Das et al<sup>[16]</sup> and Jesudanam et al,<sup>[11]</sup> 60%, 40.2%, 54.5%, 51.76% and 45.53% samples, respectively, were found to be positive by KOH and/or culture.

In our study, positivity by direct microscopy (34.71 %) was more than culture (32.65 %), similar to another studies conducted by Jesudanam et al<sup>[11]</sup> (42.4 % and 39.73 %), Lone et al in<sup>[15]</sup> (56 % and 40 %) and Shenoy et al<sup>[17]</sup> (53 % and 35 %) while, studies conducted by Suryawanshi et al<sup>[6]</sup> (43.49 % and 44.46 %), Das et al<sup>[16]</sup> (32.9 % and 49.4 %) and Kaur et al<sup>[18]</sup> (63 % and 79 %) found more positivity by culture than direct microscopy. The variation in positivity by microscopy in different places might be due to varying technical expertise in microscopic examination in different places.

Out of the 95 positive cultures, growth of non dermatophytic moulds predominated (56.84 %), followed by yeasts (26.32 %) and dermatophytes (16.84 %). The usual commonest causative agents of superficial fungal infection i.e. dermatophytes was found to be replaced by the NDMs, which were previously thought to be common laboratory or environmental contaminants. Our result was similar to study conducted by Kaur et al<sup>[18]</sup> (55 %). While in other studies conducted from various parts of India by Lakshmanan et al<sup>[19]</sup>, Veer et al<sup>[12]</sup>, Lone et al<sup>[15]</sup>, Grover et al<sup>[20]</sup>, Singh et al<sup>[21]</sup> and Kaur et al<sup>[9]</sup>, NDMs were 24.4 %, 27.9 %, 31.6 %, 34 %, 39.2 % and 44.44 %, respectively, showing a rising trend of NDMs in causing onychomycosis. However many workers have found dermatophytes as the commonest causative agent for onychomycosis in their studies viz; Veer et al<sup>[12]</sup> (60.46 %), Gupta M et al<sup>[13]</sup> (40.8 %) and Aghamirian et al<sup>[10]</sup> (50 %) while others have reported yeasts as the predominant cause viz; Suryawanshi et al<sup>[6]</sup> (47.86 %), Jesudanam et al<sup>[11]</sup> (56.74 %), Bassiri-Jahromi et al<sup>[22]</sup> (48 %) and Chadeganipour et al<sup>[23]</sup> (51 %).

In our study, most common NDM was *Aspergillus spp.* (57.41 %) followed by *Fusarium spp.* (9.26 %). Kaur et al<sup>[9]</sup>, Aghamirian et al<sup>[10]</sup>, Veer et al<sup>[12]</sup>, Gupta M et al<sup>[13]</sup> and Martinez-Herrera et al<sup>[24]</sup> also isolated *Aspergillus* as the most common NDM.

*A. nidulans* (14.74 %) and *A. glaucus* (7.37 %) were the most common isolates in the present study. *A. nidulans* was not isolated as the commonest NDM in other studies, however, Chadeganipour et al<sup>[23]</sup> and Felix Bongomin et al<sup>[25]</sup> showed *A. nidulans* as the second and English and Atkinson et al<sup>[26]</sup> as the third most common *Aspergillus* species which suggests that there is obvious increasing prevalence of *A. nidulans* in recent years similar to present study. However, most common *Aspergillus* species in other studies were different, viz; Bassiri-Jahromi et al<sup>[22]</sup> (*A. fumigatus*), Adhikari et al<sup>[27]</sup> (*A. niger*), Grover et al<sup>[20]</sup> (*A. niger*), Chadeganipour et al<sup>[23]</sup> (*A. flavus*), English and Atkinson et al<sup>[26]</sup> (*A. terreus*).

Many other NDMs isolated in our study were *Mucor spp.*, *Rhizopus spp.*, *Alternaria spp.*, *Curvularia lunata*, *Curvularia geniculata*, *Hormonema dematioides*, *Scytalidium dimidiatum*, *Natrassia mangiferae*, *Chaetomium spp.*, *Chrysosporium spp.*, *Phialemonium spp.*, *Cladophialophora boppi* and *Exophiala werneckii* (Table 2) there is an ever-growing list of moulds which have been found actively colonizing nails including the comparatively common *Aspergillus spp.* None of these fungi are keratinolytic. Therefore, all must either live on the unkeratinized intercellular cement, or must take advantage of partial denaturing of the nail keratin by pre-existing trauma or disease.<sup>[28]</sup>

Infections caused by non-dermatophytes also may not be uncommon in India. The probable reasons for the meagre reports in literature regarding such infections could be attributed to the following factors: (1) use of selective medium incorporating only cycloheximide to which non-dermatophytes are sensitive, (2) the prevailing belief that all non-dermatophytes are laboratory contaminants, (3) lack of mycological expertise.

Amongst the yeasts, *Candida albicans* (56 %) was the most common followed by *Candida tropicalis* (32 %) and *Candida glabrata* (12 %) (Table 2) which was in accordance with other studies. <sup>[6,18]</sup> While, in a study by Adhikari et al <sup>[27]</sup>, not a single case of onychomycosis due to *Candida spp.* was isolated.

Amongst the dermatophytes, *T. tonsurans* (37.5 %) and *T. mentagrophytes* (37.5 %) followed by *T. rubrum* (12.5 %) were most commonly isolated. (Table 2) Similar findings were shown by Adhikari et al <sup>[27]</sup>. However, various studies <sup>[6, 13]</sup> showed *T. rubrum* as the most common dermatophyte while R Kaur et al <sup>[18]</sup> showed *T. verrucosum* as the prevalent dermatophytic species. This suggests that causative agent may vary according to geographic location.

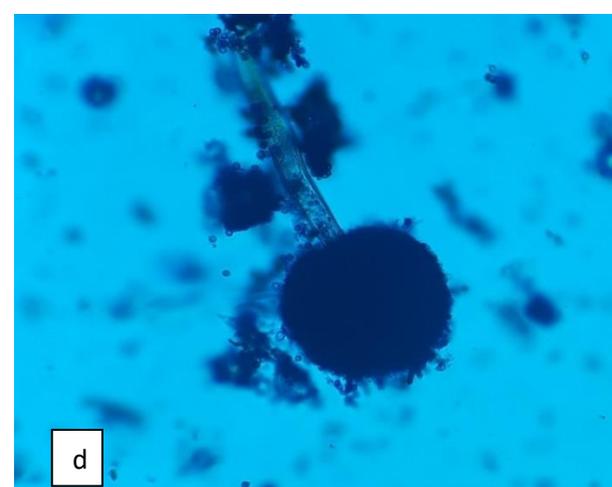
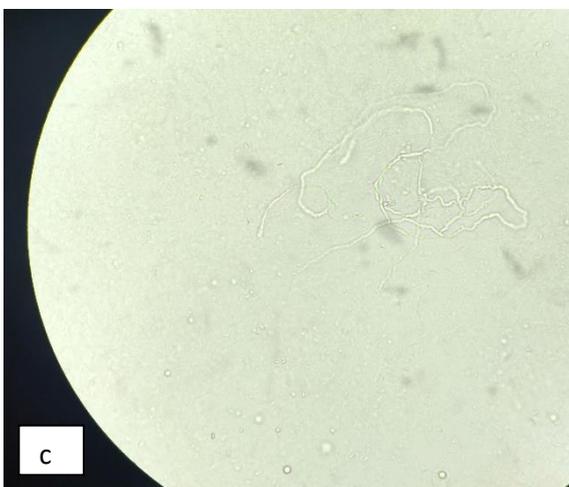
Present study shows that onychomycosis is a very common health problem in Central India, with a changing trend in etiological agents from the dermatophytes (earlier sole cause) to NDMs, which being the major cause currently. The diagnosis of onychomycosis by a NDM is difficult as compared to onychomycosis caused by dermatophytes and yeasts, as consecutive cultures from repeated nail scrapings for reliable laboratory results becomes substantial.

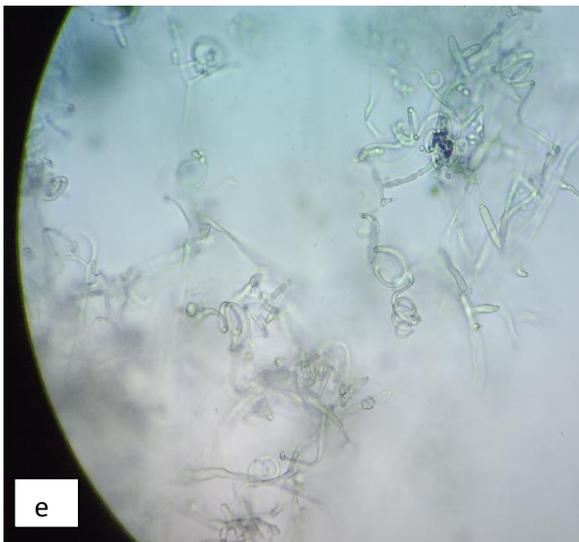
According to some authors, an untreated non-dermatophytic onychomycosis could be an important and dangerous portal of entry for deep-seated and disseminated mycosis, which are difficult to treat in immunocompromised patients, making the correct diagnosis and rapid treatment of non-dermatophytic onychomycosis essential. <sup>[29, 30]</sup> Determining the mycological cause of onychomycosis is helpful in guiding antifungal treatment and preventing complications. <sup>[31]</sup> *Aspergillus spp.* isolated from nail specimens are not susceptible to most of the topical and systemic antifungals used to treat dermatophytes. <sup>[32]</sup> Resistance to triazole antifungals occurs among the *Aspergilli*. <sup>[33]</sup>

Thus, epidemiological and mycological features and better diagnostic facilities of onychomycosis would help in decreasing the morbidity and improving the quality of life.

### Conclusion:

Present study demonstrates a shift in agents causing onychomycoses from dermatophytes and yeasts to non-dermatophytic moulds which are hard to treat. Increasing drug resistance is also a concern so prompt and efficient diagnosis is must to start early treatment and to have good prognosis. Thus, epidemiological and mycological features and better diagnostic facilities of onychomycosis would help in guiding antifungal treatment which will help to decrease the morbidity and improving the quality of life.





a) Clinical picture of onychomycosis b) SDA showing *T.mentagrophytes* c) KOH mount showing septate hyphae d) LPCB mount showing *A.niger* e) Slide culture on SDA showing spiral hyphae of *T.mentagrophytes* f) Slide culture cornmeal agar showing *Curvularia lunata*

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