



## NON DERMATOPHYTE MOULDS: NOT SO UNCOMMON AGENTS OF ONYCHOMYCOSIS

### Microbiology

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### ABSTRACT

Onychomycosis is a frequent condition seen in dermatology clinics. The causative can be dermatophyte, non-dermatophyte moulds (NDM), or yeasts. NDM can be hyaline or dematiaceous moulds with reported prevalence of 1.49% to 33.5% worldwide. This dramatic rise could be due to better diagnostic facility and increasing associated risk factors like overuse of antibiotics, chronic illness etc.

A study was undertaken in 550 patients in Department of Microbiology, Lady Hardinge Medical College. Direct microscopy of nail clips was positive 33.27% and culture was positive in 72% cases. Out of the samples cultured, yeast was isolated in 16.55%, NDM in 42.18% and dermatophytes in 9.45% samples.

Amongst the NDM, *Aspergillus* spp. was the most prevalent followed by *Alternaria* spp, *Cladosporium* spp., *Penicillium* spp and *Fusarium* spp. Males were predominantly affected (53.5%).

Culture though time consuming is important to know etiological shift in the agents of onychomycoses.

### KEYWORDS

Onychomycoses, *Aspergillus*, Non-Dermatophyte

### Introduction

Onychomycosis is a common nail disorder with a varied aetiology. It accounts for approximately 50 percent of nail diseases and 30 percent of superficial mycoses. (1,2)

Clinical presentation ranges from mild discoloration to destruction of nail plate. The implicated fungal entity is usually a yeast or dermatophyte. Increase in other fungal agents like Non Dermatophyte moulds (NDMs) as causative agents have been acknowledged in recent past. (3-5)

NDMs are fast growing, soil saprophytes and regarded commonly as laboratory contaminants. They were previously thought to be skin or environmental contaminants. The most common NDMs in the literature are: *Scopulariopsis brevicaulis*, *Fusarium* sp., *Acremonium* sp., *Aspergillus* sp., *Scytalidium* sp., etc. (5)

The prevalence of NDMs in nail infections in various parts of the world ranges between 1.49% and 33.5%. (6-8) Indian literature regarding onychomycosis is limited especially in relation to the emerging NDMs.

Various factors associated with onychomycoses are age, climate, physical activity, occupation, underlying disease etc. (9) Onychomycosis is a chronic condition requiring long treatment duration. It has a propensity to relapse due to treatment failure or noncompliance. (10)

Majority of patients seek medical help later during the course of disease. This disease is known more for the cosmetic concern leading to low self-esteem and decreased quality of life. (11)

Importance of laboratory in diagnosing onychomycosis is challenging with the currently used techniques in majority of set up. (12)

Therefore, it is essential to identify the etiological agent to ensure the appropriate diagnosis and management for such cases.

### Materials and methods

The study was conducted in the Department of Microbiology, Lady Hardinge medical College, Delhi during January 2016- December 2016. All nail samples routinely submitted with clinically suspected fungal infections were included.

A total of 550 patient's nail samples were collected. Relevant history and demographic details were taken. Nail specimen were subjected to 20 percent KOH microscopy and fungal culture. Samples were inoculated on Sabouraud dextrose agar with antibiotics (Gentamicin,

Chloramphenicol, cycloheximide) and incubated at 250 C and 370C. Culture were incubated for a period of 6 weeks before reporting as negative. (11)

All cultures grown were subjected to identification by microscopy, slide culture, temperature tolerance etc. (11)

### Results

In the duration of 12 months, 550 nail samples were submitted. All samples were processed as per standard protocol. Microscopy and culture were performed on every sample. Finger nails (340) were more commonly submitted as compared to toe nails (160). Male to female ratio was 1.14:1. Majority of females belong to 18-45 years' age (210/256). Microscopy was positive in only 183 samples (Table.1). Direct examination was either positive for yeast/thin septate hyaline hyphae with or without arthrospores/ pigmented hyphae. All KOH findings were corroborated with respective culture findings. Culture only positive samples were given significance on the account of pure growth and/or growth in duplicate tubes kept at different temperature. Isolates were photographed and stored as per feasibility (Table.2).

**Table 1. Distribution of samples**

Nail samples	Total = 550	Percentage (%)
Finger nail	340	61.82
Toe nail	160	20.09
<b>Gender</b>		
Male	294	53.50
Female	256	46.5
<b>Age</b>		
<18 years	31	5.64
18-45 years	450	81.82
>45 years	69	12.55
<b>KOH mount positive</b>	183	33.27
<b>Culture positive</b>	396	72
Yeast	91	16.55
Dermatophyte	52	9.45
Bacteria	17	3.09
NDMs	232	42.18
Mixed	4	0.73

**Table 2. Distribution of NDM isolates in samples**

Genus	No of isolates	Percentage
<i>Aspergillus</i>	123	22.4
<i>A niger</i>	44	8
<i>A flavus</i>	30	5.4
<i>A fumigatus</i>	16	2.9

<i>A terreus</i>	5	0.9
<i>A glaucus</i>	2	0.3
<i>A nidulans</i>	1	0.18
Unidentified	25	4.5
<i>Acremonium</i>	6	1.1
<i>Alternaria</i>	26	4.7
<i>Bipolaris</i>	4	0.72
<i>Chaetomium</i>	2	0.3
<i>Cladosporium</i>	15	2.7
<i>Curvularia</i>	2	0.3
<i>Fonsecaea</i>	6	1.1
<i>Fusarium</i>	7	1.3
<i>Paecilomyces</i>	5	0.9
<i>Penicillium</i>	15	2.7
<i>Syncephalustrum</i>	11	2
Unidentified dematiaceous	2	0.3
<i>Acrophialophora</i> , <i>Geotrichum</i> , <i>Rhizopus</i> , <i>Rhizomucor</i> , <i>Lichthemia</i> , <i>Phialophora</i> , <i>Scopulorpsis</i> , <i>Scedosporium</i>	1 each	0.18 each

## Discussion

Among the superficial dermatologic disorders, onychomycoses are notoriously difficult to treat. (10) Frequently there is lack laboratory diagnosis or are misdiagnosis because of inadequate facilities available.

Correct identification of etiological agent is a common problem faced by the mycologist and clinicians, experience in the field is a must. Further prevalence of commonly considered laboratory contaminants in the culture of onychomycosis samples creates great confusion. (3)

In the past, such diagnosis was given no clinical significance. But the presence of recent literature suggests otherwise. (3-5,10)

Possibility of such fungal agents, NDMs must be taken into account. Our study reveals a very high percentage of culture isolates from the NDMs group which cannot be ignored as contaminants.

The most common group of fungus in our study belong to the genus *Aspergillus* with *Asp niger* and *Asp flavus* being the most prevalent. There is enough literature to support the pathogenic potential of *Aspergillus* in onychomycosis. (13-18)

*Penicillium*, *Paecilomyces* and *Fusarium spp.* are the other hyaline group of fungi isolated in our study. Their association with onychomycosis have been reported by *Bandh et al*, *Pontini P et al* and *Ranawaka RR et al* respectively. (19-21)

Among the dematiaceous group, *Alternaria* and *Cladosporium* were commonly identified. These fungi were shown by *Lone R* and *Lestringant GG et al.* (11,22) Various fungal agents were reported only once in our sample size but their importance can be accounted as they have been documented to be causative agents of onychomycosis. (23,24)

In our study, 53.5 % patients with onychomycosis were males and 46.5% were females, with a male female ratio of 1.14:1. This finding is in concordance with *Lone R Et al.*, *Garg et al.*, and *Veer et al.* (11,25,26)

Some studies show more prevalence females possibly because of more wear and tear of nail due to constant friction and contact with water. (27,28)

According to age group, most commonly affected belonged to 18 to 45 years of age. This finding was similar to studies by *Adhikari et al* and *Lone R et al.*, where similar age groups were frequently reported to have the disease. (11,29)

In contrast, *Velez et al.*, and *Mercantini et al.*, reported higher prevalence among adults who were over 50 years of age. (28,30)

Young individuals are more prone to nail infection due to constant micro trauma to the nails predisposing to invasion by fungus. And in case of elderly, underlying comorbidities could be a reason for presentation of disease.

Finger nails were more commonly involved than toe nails. *Aghamirian MR et al.*, *Grover S et al.*, and *Lone R et al.* also reported frequent involvement of fingers as compared to toe nails. In contrast *Kaur R et al.* reported toe nails to be regularly affected with onychomycosis. (14,31,32)

The reason for concern in fingernail infection could possibly act as a bias on part to the patient to seek medical attention earlier than toe nail disorder. (33)

Direct microscopy positive using the convention 20 % KOH method gave a positivity of 33.27 percent. This finding is less as compared to that reported by *Bukowski P et al.* and *Zanardi D et al.*, which showed more than 44 and 100 percent of positive samples respectively. (34,35) Use of Microscopic methods alone is usually relied for the fungal diagnosis in many set up without the option for culture.

Such practices are frequent in many public and private clinics because of ease and logistics.

Fungal culture requires prolonged incubation and expertise for reporting. Drug therapy is routinely started based on the initial microscopy result only. Faster result of direct microscopy usually given by technical staff rather than experienced mycologists can have its demerits.

Sensitivity and specificity of such methods relies on correct sampling technique and reagents used. Use of special stains like PAS and Calcofluor with fluorescence microscopy can enhance the potential of initial step of diagnosis. Significant difference in the microscopy result has been shown by different authors on using varied techniques like KOH, Calcofluor, histology etc. (36)

Treatment of NDMs needs special attention as they are difficult to cure. Resistance to antifungals is on the rise with more and more data on genus and species specific resistance profiles being reported. (37,38)

The problem with routine antifungal susceptibility testing is multifactorial: lack of facility, and standardised methods for majority of moulds. Knowledge of causative agent and specific drug therapy for individual patient can potentiate the importance of fungal culture and correct identification. Precise role of NDMs as pathogen or contaminants can be questioned. But the presence of these agents in such a huge proportion with the availability of supporting literature allows us to consider their role in onychomycosis.

Limitation of the current study was lack of follow up of all patients and decreased positivity in direct microscopy. The reason for the latter could be lack of special staining methods like Calcofluor in our set up.

Further studies are required for supporting our findings like molecular techniques using PCR, MALDI-TOF etc. for direct detection of fungal agent from sample.

In conclusion, identification of fungal agent using culture is a must and NDMs to be taken into consideration if other possible agents are ruled out.

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