

Prevalence of Onychomycosis by Non Dermatophyte Molds in a Tertiary Care Hospital in Kolkata , West Bengal, India

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Background and study aim:

Onychomycosis is infection of dermatophytes and Candida sp, and unusually by non-dermatophyte molds (NDM). These NDM are opportunistic fungus seen in plant & soil. Literature on nail mycosis classifies the onychomycosis as Distal and Lateral Subungual, White Superficial, Proximal Subungual, Total Dystrophic and secondary onychomycosis. The aim of this study is to determine the prevalence of non-dermatophyte Molds causing onychomycosis.

Patients and Methods: Samples from clinical nail mycosis cases were studied in our Mycology dept. over a period of 1 year from January, 2023 to December, 2023. After informed consent, history-taking and patients' examination, the samples were evaluated by KOH wet mount, culture in SDA and SDCA media at 25°C and 37°C, Slide Culture, LPCB wet mount etc. The data were analyzed

using counts and percentages in MS Excel.

Results: Out of 114 positive culture 32 cases (28.07%) of onychomycosis was by NDM. The most affected age group of NDM Onychomycosis ranged from 25-60 years (22 patients, 68.75%) and females were more commonly affected (18 cases, 56.25%). The risk factors mostly may be poor socio-economic status with occupation of manual labourers. Total NDM nail mycosis diagnosed were Talaromyces spp - 8 cases (25%), Fusarium spp.-6 cases (18.75%), Mucor spp.-4 cases (12.5%), Rhizopus spp. - 4 cases (12.5%) and 2 cases(6.25%) each of Medicopsis romeroi, Hortaea werneckii, Trichosporon spp., Paecilomyces spp. & Bipolaris spp.

Conclusions: Onychomycosis by unusual fungus were found in 28% cases and Total Dystrophic Onychomycosis was present in 62.5% cases. The most frequent NDM were : Talaromyces spp. and Fusarium spp.

INTRODUCTION

Onychomycosis is a major cause of nail dystrophy caused by dermatophytes, Candida and Non Dermatophyte Molds (NDM). Different species of molds have been diagnosed from nails [1]. Though Dermatophytes are the major causative agents of Onychomycosis, Non Dermatophyte Molds (NDM) are also gaining importance as emerging causes of Onychomycosis in recent days.

NDM Onychomycosis is often difficult to diagnose as they have been

considered nail contaminants. Though various diagnostic methods are there to diagnose NDMs however, repeated isolation by culture in laboratory is recommended to confirm NDMs as pathogens of Onychomycosis. Increased incidence of Onychomycosis by NDM and mixed infection (both Dermatophytes with Non Dermatophyte Molds) onychomycosis have been observed in recent days recommends towards more accurate clinical diagnosis along with mycological tests [2].

Opportunistic NDMs are seen in nature as plant pathogens and soil saprophytes. They are fast growing with universal presence and are often un-noticed laboratory contaminants. Different literature revealed that the most commonly isolated species of NDM are *Scopulariopsis brevicaulis*, *Fusarium* sp., *Acremonium* sp., *Aspergillus* sp., *Scytalidium* sp., and *Onychocola canadensis* [3].

A wide group of NDM species and some saprophyte yeasts may also affect the nail plate directly. These include some species of the genera *Alternaria*, *Curvularia*, *Trichosporon* and *Hendersonula* [4].

It is difficult to establish the pathogenicity of opportunistic, NDMs on nails as in all opportunistic infection. There are always a number of criteria that need to be fulfilled. The culture must be free from dermatophytes and the NDMs should be observed in the microscopic examination using 10-40% potassium hydroxide (KOH) [5].

Onychomycosis is the most difficult to treat superficial mycosis. It is chronic infection that is prone to relapse. Therefore, it is important to identify the causative agent to ensure that the appropriate treatment is employed for each case [6].

PATIENTS AND METHODS

All samples from onychomycosis cases sent by different Clinical departments of our hospital for Fungal culture in our Mycology laboratory of Department of Microbiology, NRS Medical College, were studied over a period of one year from January 2023 to December 2023 after meeting all ethical Consideration as per declaration of Helsinki .

After informed consent, history-taking and patients' examination, , samples were collected from both nail plates & subungual debris as per clinical presentation; the collected samples were evaluated by KOH wet mount and Fungal culture in Sabouraud's Dextrose Agar (SDA) and Sabouraud's Dextrose Chloramphenicol Agar (SDCA) at 37°C and 25°C followed by study of Culture growth by their colony morphology, presentation, pigmentation of reverse & obverse surfaces followed by LPCB teased mount and Slide culture where ever necessary. Inoculation into Blood Agar plates done in some cases to

demonstrate Schizogenous yeast conversion. Comparison of findings of KOH wet mount with culture and Repeat culture from samples were also done to establish that the isolates were pathogenic fungi not laboratory contaminants [7].

The received data was summarized using counts and percentages and other statistical methods in Microsoft Excel software as appropriate.

RESULTS

Among the 114 samples of culture positive Onychomycosis, 32 cases (28.07%) of Non Dermatophyte Molds (NDMs) were detected to cause Onychomycosis [Chart: 1].

Gender-wise distribution of NDM Onychomycosis revealed females were more commonly affected (22 cases, 68.75 %) than males (10 cases , 31.25%) [Chart: 2].

The most common age group of NDM Onychomycosis were among 25 years to 60 years (22 patients, 68.75%) followed by 16-24 years (5 patients , 15.62%) followed by more than 60 years group (3 patients , 9.37%) and lastly 6-15 years age group (2 patients , 6.25%) [Table :1].

Among these NDM onychomycosis, the finger nails were found to be more affected with 40.63% (13 cases) followed by toe nails of 34.37% (11 cases) and both Toe & Finger nail involvement was 25.00% (8 cases) [Table: 2]. Clinical type-wise distribution of the 32 NDM Onychomycosis revealed 20 patients (62.50%) were of Total Dystrophic Onychomycosis (TDO), 11 (34.37%) were White Superficial Onychomycosis (WSO), 2 (6.25%) were Distal & Lateral Subungual Onychomycosis (DLSO) and we did not get any NDM isolate from Proximal Subungual Onychomycosis (PSO). In almost all clinical types of Onychomycosis females were found to be more affected than males [Table : 3].

The demographic data of the study population revealed that the patients were mostly from poor socio-economic background; Some adult females were house-wives and some were working as domestic help and some were working in brick manufacturing units. Males were mostly farmers, Some were gardeners and few were garage workers.

Among the 32 unusual NDM isolates causing onychomycosis 8 cases (25%) were *Talaromyces* spp. [Figure: 1], 6 cases (18.75%) were *Fusarium* spp. [Figure: 2], 4 cases (12.5%) were *Mucor* spp. [Figure: 3], 4 cases (12.5%) were *Rhizopus* spp. [Figure: 4], and 2 cases (6.25%) each of

Medicopsis romeroi [Figure: 5], *Hortaea werneckii* [Figure: 6], *Trichosporon* sp [Figure: 7], *Paecilomyces* spp.[Figure: 8] and *Bipolaris* spp.[Figure: 9A &9B].

Chart - 1 : Percentage of Dermatophyte & Non Dermatophyte Molds Onychomycosis (n=32)

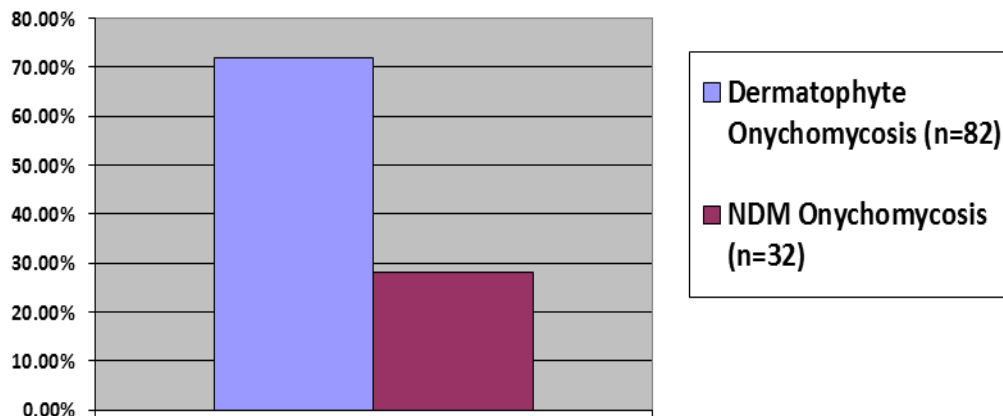


Chart – 2 : Gender-wise Distribution of NDM Onychomycosis (n=32)

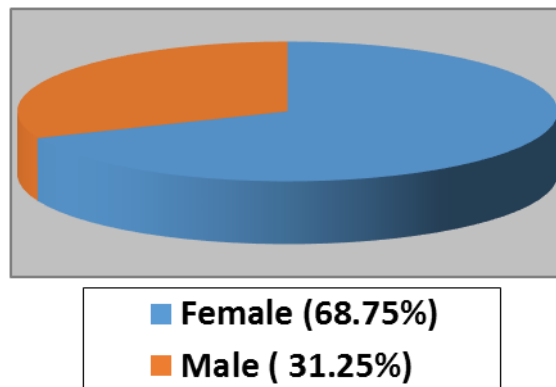


Table – 1 : Age-wise Distribution of NDM Onychomycosis (n=32)

Age-group	Number of cases of NDM Onychomycosis (n=32) [100%]	
	Male	Female
< 1 month	0	0
1 Month to < 1 year	0	0
1 year to 5 years	0	0
6 years to 15 years	2 (6.25%)	0
16 years to 24 years	2 (6.25%)	3 (9.38 %)
25 years to 60 years	4 (12.50%)	56.25%) -
>60 years	2 (6.25%)	1 (3.12 %)
Total 32 (100%)	10 (31.25%)	22 (68.75%)

Table – 2 : Site-wise Nail involvement in NDM Onychomycosis

Site of Involvement	Non Dermatophyte Molds Isolated (n=32)
Finger nails only	13 (40.63%)
Toe nails only	11 (34.37%)
Both Toe & Finger nails	8 (25.00%)
Total	32 (100%)

Table – 3 : Onychomycosis Clinical Type-wise and Gender-wise Distribution OF NDM Isolates (n=32)

Clinical Types of Onychomycosis	Non Dermatophyte Molds Isolated (n=32)	
	Male (n=10) [31.25%]	Female (n=22) [68.75%]
Total Dystrophic Onychomycosis (62.51%)	6 (18.76%)	14 (43.75 %)
White Superficial Onychomycosis (31.25%)	3 (9.38 %)	7 (21.87 %)
Distal & Lateral Subungual Onychomycosis (6.24%)	1 (3.12 %)	1 (3.12 %)
Proximal Subungual Onychomycosis (0.00%)	0	0

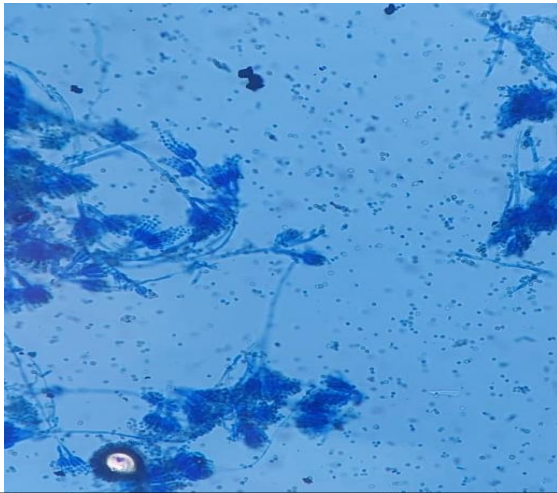


Figure :1 LPCB mount showing conidiophore with terminal production of primary metulae and secondary phialides from which chains of spherical conidia are produced forming broomstick/brush-border appearance; Inoculation on blood agar induced schizogenous yeast formation, suggestive of *Talaromyces spp.*

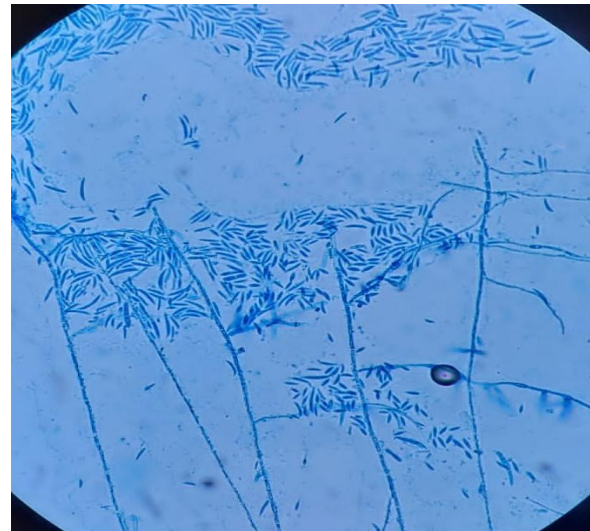


Figure:2 LPCB mount showing long, sickle-shaped, multicelled macroconidia, transverse septa separating the cells from one another within the macroconidia, suggestive of *Fusarium spp.*

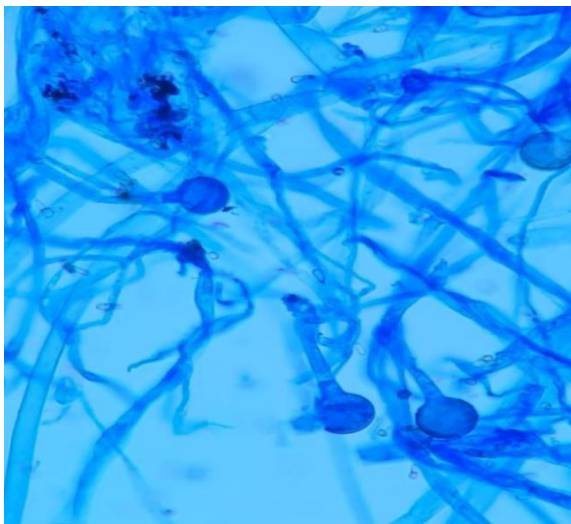


Figure: 3. (A)&(B): Mouldy colony having salt and pepper appearance, (E): 40% KOH mount showing presence of fungal hyphae (C),(D)&(F): LPCB mount showing ribbon like aseptate hyaline hyphae with acute angled branching and no rhizoids, suggestive of *Mucor spp.*



Figure: 4 LPCB mount showing ribbon like aseptate hyaline hyphae with acute angled branching, presence of nodal rhizoids and sporangiophores terminating in globe-like columella within the sporangia that contains sporangiospores, suggestive of *Rhizopus spp.*

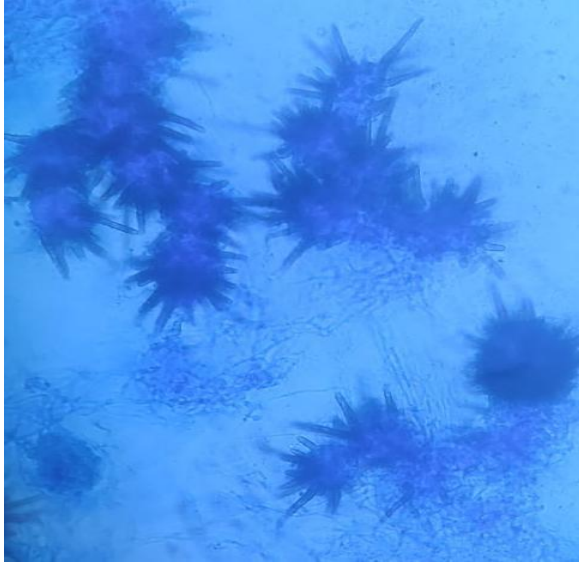


Figure: 5 . LPCB mount showing typical pycnidia with septae suggestive of *Medicopsis romeroi*

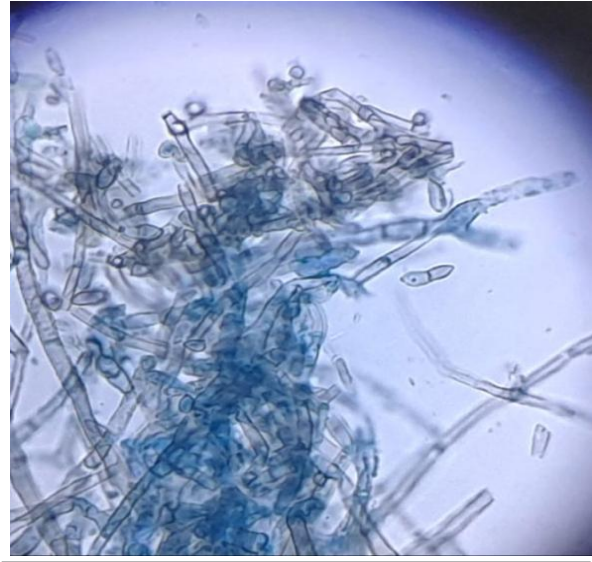


Figure: 6 .LPCB mount showing elongated double yeast cells separated by a distinct deeply staining transverse bar (annelide) suggestive of *Hortaea werneckii*

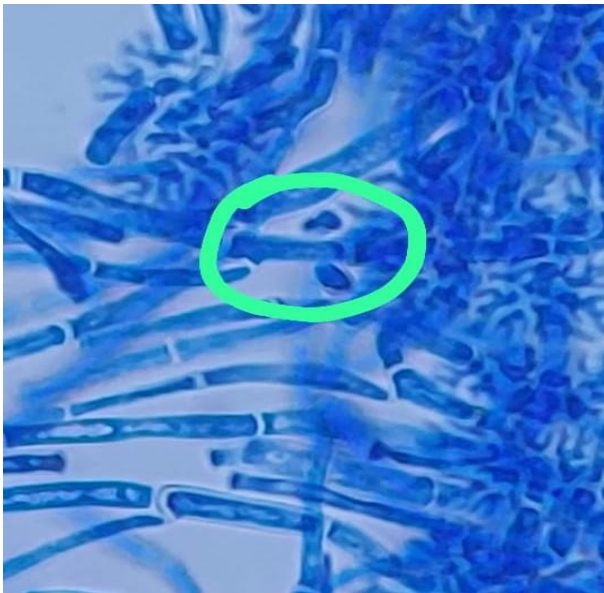


Figure: 7 .LPCB mount of Slide culture showing illustrating arthroconidia , one of which presents the characteristic budding blastoconidia from two corners resembling "Rabbit Ear" suggestive of *Trichosporon* spp.

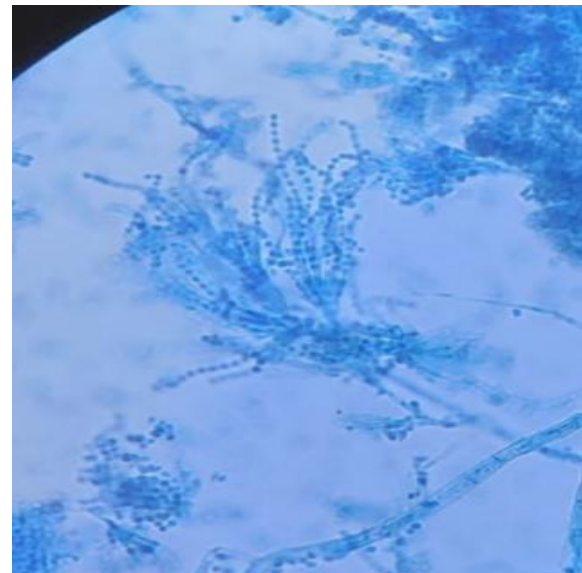


Figure: 8 .LPCB mount showing long tapered phialides from the tips of which chains of elliptical conidia are produced, suggestive of *Paecilomyces* spp.

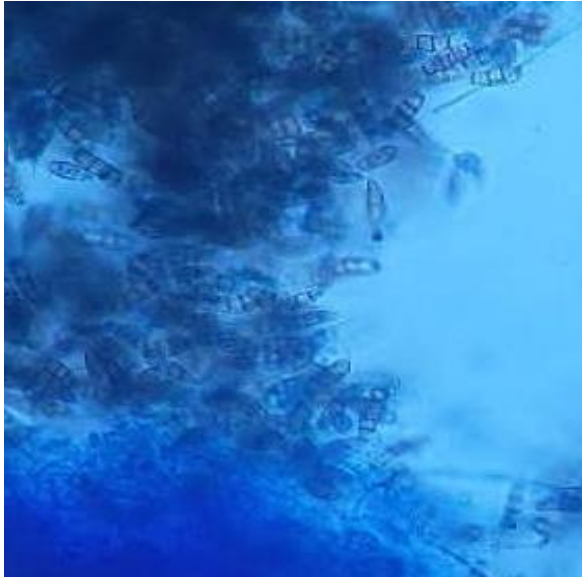


Figure:9A LPCB mount showing multicelled, elliptical to oval, thick-walled macroconidia with smooth surfaces, with incomplete septations (dystoseptate), suggestive of *Bipolaris spp.*



Figure: 9B Colony suspension prepared from *Bipolaris spp.* with normal saline (kept in a sterile petri dish) at 25°C for 24 hours to check for germ tube formation revealing Germ tubes formation arising from both (i.e. bi) end (i.e. polar) cells of the macroconidia, which grow in parallel with the long axis of the cell.

DISCUSSION

In our study of Onychomycosis we found 28.07% of NDM isolates among culture positive onychomycosis which is nearly corroborated with a study by Pukhrambam PD et. al. [8] in RIMS, Imphal of India revealing 31.3% of NDM onychomycosis isolates and also closely in accordance with a study by Raghendra KR. et. al. [9] in South East Rajasthan of India showing 35.33% of the same.

Among the NDM Onychomycosis of our study the females were more affected with 68.75% which is in tandem with studies by Marinez-Herrera EO. et. al. [10] in Guatemala and a study by S Hilmioglu Polat et. al. [11] in Turkey revealing 65.6% and 76% of female preponderance of NDM onychomycosis respectively. The most affected age group of NDM Onychomycosis in our study was 68.75% from (25-60) years followed by 15.62% from (16-24).

years age group, 9.37% from more than 60 years age group and 6.25% from (6-15) years age group; All these findings are in close accordance with a study by Raghendra KR et. al. [9] in South East Rajasthan showing their mean affected age group of (34.6+ 18) years. Similar demographic features are also seen in a study by Marinez-Herrera EO. et. al. [10] in Guatemala which revealed (41-65) year age group to be mostly affected group of NDM Onychomycosis and closely corroborates with the observation of our present study. Site of involvement-wise, the finger nails alone were found to be more affected with 41% followed by toe nails of 35% which are very closely corroborated with the study by Raghendra KR et.al. [9] showing involvement of 38% finger nails & 33.33% of toe nails. both Toe & Finger nail involvement was 25% (8 cases) in our study which is in tandem with a study by Raghendra KR et.al. [9] with 15% cases.

The higher incidence of finger nail onychomycosis may be due to occupation of domestic help or farming which needs active involvement of hands in moist conditions and slight lower incidence of toe nail involvement may be due to open footwear habit and lesser concern of feet & toenails in those occupation in our present study [9].

Clinical type wise NDM Onychomycosis showed 62.50% were of Total Dystrophic Onychomycosis (TDO) which is highest in our present study and closely corroborates with a study by Raghavendra KR et.al.[9] showing TDO to be the major contributor with 46% in their study. The second major contributor of NDM Onychomycosis was 34.37% of White Superficial Onychomycosis (WSO) followed by 6.25% of Distal & Lateral Subungual Onychomycosis (DLSO) in our study; this observation was not in tandem with other studies by Raghavendra KR et.al. [9], revealing WSO of 7.33% & DLSO of 34.6% and another study by Marinez-Herrera EO. et. al. [10] revealing DSLO of 62.5% & TDO of 37.5%. The different geographical location and variety of occupation may be responsible for these variations in occurrence.

In our study *Talaromyces* spp. was the major NDM isolate with 25% contribution among the 32 unusual NDM isolates causing onychomycosis followed by 18.75% of *Fusarium* spp., 12.5% were *Mucor* spp., 12.5% were *Rhizopus* spp., 6.25% each of *Medicopsis romeroi*, *Hortaea werneckii*, *Trichosporon* spp, *Paecilomyces* spp., & *Bipolaris* spp.; A study by Pukhrambam PD. et.al. [8] revealed that *Aspergillus* spp. was commonest NDM causing Onychomycosis followed by *Penicillium* spp. 14%, *Acremonium* spp. 4.8%, *Fusarium* spp. 1.8%, *Curvularia* spp. 1.6%, *Alternaria* spp. 1.4%, *Scopulariopsis* spp. 1%, *Cladosporium* spp. & *Nigrospora* spp. 0.8% each, *Mucor* spp. 0.4%, and *Paecilomyces* spp., *Pseudoallescheria* spp., *Rhizopus* spp., *Verticillium* spp. & *Exophiala jeanselmei* with 0.2% each. These observation though not much in agreement as per their incidence with our study but many NDM Mold species were in common to be the cause of Onychomycosis.

Another study by Sarkar M. et. al. [12] in 2014-15 in Kolkata revealed *Fusarium* as the major NDM isolate to cause onychomycosis followed by *Aspergillus* spp. is partially in tandem with

our study findings as *Fusarium* was the second most common isolate after *Talaromyces* spp. in our present study. All these observations prompt to the fact that NDM isolates causing Onychomycosis vary with geographical location and passage of time.

Occurrence of NDM Onychomycosis by unusual pathogen is a rising trend and in India relatively less work has been done on the onychomycosis as compared to western countries[13,14]. The evolving role of non-dermatophytic molds has added a new dimension to the clinical patterns of onychomycosis as they are relatively resistant to conventional antifungals [14].

CONCLUSION

The study showed that non-dermatophytic molds were responsible for nearly 28% of onychomycosis cases attending in different clinical out patient departments of our hospital. Dystrophic Onychomycosis was present in 62.5% cases. The two most common NDM isolates were *Talaromyces* spp & *Fusarium* spp. As molds are common laboratory contaminants, repeat culture isolation of the same species and matching the findings with KOH wet mount from successively taken nail samples are ideal and should be carefully evaluated to diagnose a "NDM Onychomycosis". Changing trends of causative agents by unusual Non Dermatophyte Molds causing Onychomycosis needs continuous study and evaluation to manage these patients with empirical therapy based on available data.

Author contribution: We declare that all listed authors have made substantial contributions to all of the following three parts of the manuscript:

- research design, or acquisition, analysis or interpretation of data;
- drafting the paper or revising it critically;
- approving the submitted version.

We also declare that no-one who qualifies for authorship has been excluded from the list of authors.

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Conflict of interest : None

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department of NRS Medical College, Kolkata, West Bengal, India.

Ethical Considerations :The study was conducted after meeting all ethical Consideration as per declaration of Helsinki .

RESEARCH HIGHLIGHTS:

1. The most prominent research highlight of our study revealed that the predominant Non Dermatophyte Mold causing onychomycosis is Talaromyces spp. which has hardly been observed in any other related available study done on NDM Onychomycosis.
2. In addition we have observed 2 more unusual Non Dermatophyte Molds causing Onychomycosis namely *Medicopsis romeroi* & *Hortae werneckii* which is also rare and hardly observed in any other related studies done on NDM Onychomycosis.
3. In our study the females were found to be more affected by NDM Onychomycosis in comparison to male population.
4. Repeated fungal culture was done to establish the pathogenic fungus and to exclude the laboratory contaminants.

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