

Original article:**Non-dermatophytic moulds and yeasts as agents of onychomycosis in a Malaysian medical centre**Ding CH¹, Rahman MM², Tzar MN³, Yusoff H⁴, Satim H⁵**Abstract:**

Objective: Onychomycosis can be caused by numerous fungi, with geographical and temporal factors influencing the prevalence of the various aetiological agents. We set out to identify and classify the various fungi cultured from the nail clippings of patients diagnosed with onychomycosis in UKM Medical Centre, Malaysia. **Methods:** This retrospective study involved cases of onychomycosis diagnosed from January 2013 to July 2014. For each fungus, mycological diagnosis was achieved by direct microscopic examination of the nail clipping(s) followed by morphological identification of the fungus following culture on various artificial media. **Results:** A total of 180 fungal isolates were cultured from the nail clippings of 146 different patients. Non-dermatophytic moulds accounted for most of the fungal isolates (59.8%), followed by yeasts (35.7%) and dermatophytes (4.5%). Overall, *Candida* was the most frequently isolated fungal genus and *Aspergillus* was the most frequently cultured mould genus. Out of the three dermatophyte genera, two (*Microsporum* and *Trichophyton*) were isolated. **Conclusion:** In our centre, non-dermatophytic moulds and yeasts are a lot more prevalent as causative agents of onychomycosis than dermatophytes.

Keywords: Dermatophyte; Non-dermatophytic mould; Nail clipping; Onychomycosis

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Introduction

Onychomycosis (plural: onychomycoses) is a common condition, making up approximately half of all nail disorders¹. Dermatophytes have previously been reported to be the dominant aetiological agents in onychomycosis^{1,2}. All three dermatophyte genera (*Trichophyton*, *Epidermophyton* and *Microsporum*) are implicated in onychomycosis. However, the role of non-dermatophytic moulds (NDMs) in onychomycosis has been recognized as early as the 1960s³. A review of the recently published medical literature has shown that NDMs are emerging as causative agents of onychomycosis and in some reports, have even overtaken dermatophytes as the most prevalent causes of onychomycosis⁴.

Onychomycosis, like other cutaneous (i.e. hair and

skin) infections, do not result in a significant degree of morbidity. However, they can result in cosmetic disfigurement, thereby potentially impacting quality of life. Dermatophytes have the capability to produce keratinase, allowing them to metabolise and live on human keratin⁵. NDMs on the other hand, are not keratinolytic but they are able to live on the non-keratinized intercellular cement or take advantage of previous keratin degradation by dermatophytes, trauma, or a pre-existing nail pathology⁶. Some NDMs (e.g. *Fusarium* sp.) may even have intrinsic resistance properties⁷, which would limit or complicate therapeutic options.

Several studies have shown that the types of fungi isolated from dermatological specimens can vary geographically⁸ as well as temporally⁹. This study

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aims to identify the various aetiological agents causing onychomycosis among patients of Universiti Kebangsaan Malaysia Medical Centre (UKMMC). It is hoped that this local data can be of use in designing empirical treatment regimes for patients with onychomycosis. For instance, griseofulvin is effective against dermatophytes, with no activity against *Candida* spp. or any of the NDMs.

Materials and Methods

This retrospective study involved the analysis of laboratory results from patients diagnosed with onychomycosis from January 2013 to July 2014. The clinical specimens were nail clippings. If the same fungus was isolated from more than one specimen from the same patient, only the first specimen yielding the fungus was included for analysis. Patients whose samples were submitted in a way which was unsuitable for culture (e.g. in formalin) were excluded.

Microscopic examination was first carried out by placing the nails on glass slides with 1-2 drops of 10% potassium hydroxide (KOH) to lyse the keratin. The slides were then heated briefly over a flame and left to react with the KOH for at least 30 minutes (or sometimes overnight) before using a light microscope to visualize fungal elements. All specimens were cultured on Sabouraud dextrose agar, Sabouraud dextrose + chloramphenicol agar, and Mycobiotic agar. Cultures were incubated at 30°C and examined regularly for fungal growth. Culture plates without growth within the inoculation areas after 30 days were discarded.

For moulds, colonial morphology was examined in a biosafety class II cabinet. The obverse side of the colony was examined for its colour, pigmentation and texture whereas the reverse side was examined for colour. Following this, a smear or impression of the colony was done and stained with Lactophenol cotton blue (LPCB). Using a light microscope, hyphal and conidial characteristics were examined as described by Larone¹⁰.

For yeasts, the colonies were examined for colour and texture. A urease test was also performed to differentiate *Candida* (urease negative) from other yeast genera (mostly urease positive). The yeasts were subcultured onto chromogenic agar and cornmeal agar (CMA). On chromogenic agar, different species of yeast produced different coloured colonies, allowing a presumptive identification to be made. Identification was done according to the microscopic CMA features as described by Larone¹⁰.

Fungal isolates were grouped as yeasts, dermatophytes

and NDMs. NDMs were further sub-grouped according to features of their hyphae into hyaline moulds, dematiaceous moulds and zygomycetes. However, if a culture containing a dermatophyte had mixed growth, the co-existence of any NDM or yeast in the same culture was disregarded and only the dermatophyte was included for analysis. Prior to submission, this study was approved by UKM's Research Ethics Committee.

Results

Nail clippings from 146 different patients were cultured and a total of 180 fungal isolates were cultured from these clippings (Table 1). The isolation rate of dermatophytes from nail clippings was only 4.5%, with NDMs accounting for 59.8% and yeasts 35.7%. We managed to culture yeasts belonging to 4 different genera and moulds from 21 genera (2 dermatophyte + 19 non-dermatophyte genera). Overall, *Candida* was the most frequently isolated fungal genus, with its members contributing to a quarter of all the fungi cultured. Among the mould genera, *Aspergillus* had the highest isolation rate, with its members accounting for nearly a fifth of all the fungi cultured. Out of the three known dermatophyte genera, only *Microsporum* and *Trichophyton* were isolated.

Fungal isolates	Number	(%)
YEASTS		
<i>Candida albicans</i>	16	(8.9)
Non-albicans <i>Candida</i>	30	(16.7)
<i>Hortae werneckii</i>	1	(0.6)
<i>Rhodotorula</i> spp.	5	(2.8)
<i>Trichosporon</i> spp.	12	(6.7)
DERMATOPHYTES		
<i>Epidermophyton</i> spp.	0	(0)
<i>Microsporum</i> spp.	1	(0.6)
<i>Trichophyton</i> spp.	7	(3.9)
NON-DERMATOPHYTIC MOULDS		
Hyaline moulds		
<i>Aspergillus</i> spp.	44	(24.4)
<i>Chrysosporium</i> spp.	1	(0.6)
<i>Fusarium</i> spp.	16	(8.9)
<i>Malbranchea</i> spp.	5	(2.8)
<i>Onychocola canadensis</i>	1	(0.6)
<i>Penicillium</i> spp.	8	(4.4)
<i>Scedosporium</i> spp.	1	(0.6)

Fungal isolates	Number	(%)
Non-sporulating hyaline moulds	9	(5)
Dematiaceous moulds		
<i>Aureobasidium pullulans</i>	1	(0.6)
<i>Chaetomium</i> spp.	1	(0.6)
<i>Cladosporium</i> spp.	3	(1.7)
<i>Curvularia</i> spp.	3	(1.7)
<i>Madurella</i> spp.	3	(1.7)
<i>Phialeacromonium</i> spp.	1	(0.6)
<i>Phialophora</i> spp.	1	(0.6)
<i>Scytalidium</i> spp.	5	(2.8)
<i>Stemphylium</i> spp.	1	(0.6)
Zygomycetes		
<i>Absidia</i> spp.	1	(0.6)
<i>Rhizopus</i> spp.	2	(1.1)
<i>Syncephalastrum</i> spp.	1	(0.6)
Total	180	(100)

Table 1: Fungi cultured from nail clippings

Discussion

We report a high isolation rate of NDMs (nearly 60%) from our nail specimens. We acknowledge that diagnosing NDM onychomycosis is difficult because these moulds may also be isolated as both specimen and laboratory contaminants. There is a set of diagnostic criteria used to diagnose onychomycosis, which includes the identification of the NDM in the nail by microscopy using KOH preparation, isolation of NDM in culture, exclusion of a dermatophyte in culture, repeated isolation of the NDM in culture, NDM inoculum counting, and histological evidence of the NDM¹¹. Most studies utilize at least three criteria¹¹, and thus we have chosen to apply the first three criteria in our study.

At an isolation rate of 24.4%, *Aspergillus* sp. was the most frequently isolated NDM in our study. Other investigators have also reported high (if not higher) aspergilli isolation rates from nail specimens. An Iranian study reported that aspergilli were isolated from 29.2% of their positive specimens¹² and an Indian study reported an isolation rate of 30%⁴. On the isolation of *Candida* spp. from nails, our isolation rate of 25.6% is close to that published by a Pakistani study, in which 30.7% of onychomycosis cases were positive for *Candida* spp.² Indian investigators have found a much higher occurrence of *Candida* spp. (40.4%) in their onychomycosis cases¹³.

It is likely that cultural and/or economic influences play a role in the high isolation rate of aspergilli (environmental moulds) and *Candida* spp. (normal human flora) from nail specimens. Most of the cases in the Indian study with the high rate of *Aspergillus* onychomycosis were agricultural workers⁴. Due to the ubiquitous nature of aspergilli, working in agriculture would result in constant exposure to this mould. Patients with low incomes and working as housemaids are likely to have candidal onychomycosis because they are required to clean and wash a lot, which chronically exposes their nails to detergents and water². There is little temporal variation in the prevalence of both *Aspergillus* and *Candida* spp. as agents of onychomycosis within our institution as a similar study on nail specimens collected from 2008 - 2010 produced figures of 27.3% and 27.4%, respectively¹⁴.

Knowing the causative agents of onychomycosis is not merely of academic interest. It has been reported that the classical systemic treatments for onychomycosis caused by dermatophytes (e.g. itraconazole, fluconazole and griseofulvin) may fail when the nail infection is caused by NDMs¹⁵. There is also a recommendation for topical amphotericin B to be considered as a first-line treatment of NDM onychomycosis⁶. Amphotericin B is also efficacious against yeasts, and may be desirable when a significant proportion of the yeasts are non-albicans *Candida*, which are known to have a higher resistance rate to azoles¹⁶. In our study, only one third of the *Candida* spp. isolated was *Candida albicans*, with the remaining two thirds being a mixture of non-albicans *Candida*.

In conclusion, dermatophytes are no longer considered the most common aetiological agents of onychomycosis in our centre. NDMs and yeasts are now more prevalent and the empirical treatment regimes of onychomycosis should include an antifungal agent (e.g. amphotericin B) with good activity against these fungi.

Conflicts of Interest

The authors have no conflicts of interest to declare

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