ORIGINAL ARTICLE

Non-dermatophyte Moulds as Emerging Pathogens of Onychomycosis among Patients Attending Mansoura University Hospital, Egypt

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ABSTRACT

Background: *Onychomycosis is fungal infection of nails. Dermatophytes, Candida species, and non-dermatophyte moulds are the commonest causes of onychomycosis.* ***Objectives:*** *This study assessed the frequency of non-dermatophyte moulds causing onychomycosis and their antifungal susceptibility.* ***Methodology:*** *Our study included106 nail specimens obtained from patients attending Mansoura University Hospital from October 2019 to March 2021. All samples were examined by standard mycological methods.* ***Results:*** *Onychomycosis was detected in 86 nail specimens. Dermatophytes were isolated from 7 cases (6.6%), non-dermatophyte moulds from 62 cases (58.5%), Candida species from 17 cases (16.0%), and the remaining 20 cultures were negative for fungal growth. Among non-dermatophyte moulds the predominant isolates were Aspergillus fumigatus (25.5%), Aspergillus niger (17.9%), Aspergillus flavus (3.8%), Alternaria alternata (2.8%) and Penicillium species (2.8%). The non-dermatophyte moulds were highly sensitive to itraconazole and ketoconazole.* ***Conclusion:*** *Non-dermatophyte moulds are a common cause of onychomycosis that cause failure of therapy and should be considered in cases of onychomycosis.*

INTRODUCTION

Onychomycosis is an invasive fungal infection of the nail which causes dystrophic changes in its color, texture, and structure. About 50% of all nail disorders and 30% of cutaneous fungal infections are due to onychomycosis[1].

Onychomycosis affects toenails more than fingernails which may be attributed to the repeated trauma, Diabetes Mellitus (DM), corticosteroids and other immunosuppressive drugs, poor peripheral circulation, smoking, poor foot care, tinea pedis and positive family history. Most of these factors are more common with increased age which explains the high prevalence of onychomycosis in the old age compared with the young age[2].

Meanwhile, the fingernails onychomycosis is commonly seen in females as they are more likely exposed to water and chemical detergents during daily domestic work. Agricultural workers, laborers, fishmongers, athletes and launderers are also more liable to onychomycosis[3].

Fungi causing onychomycosis vary with the geographical distribution and differ from one country to another according to temperature and lifestyle of these countries[4]. Dermatophytes particularly *Trichophyton rubrum* and *Trichophyton mentagrophytes* represent 90% of toenails fungal infection and 50% of fingernail onychomycosis[5].

Candida species are second in frequency to dermatophytes as causative agents of onychomycosis. They constitute 10%-32% of toenail onychomycosis and 51%-70% of fingernail infections, either as a primary pathogen or in combination with dermatophytes or non-dermatophyte moulds (NDMs)[6].

Non-dermatophyte moulds are saprophytic non-keratinolytic fungi that live in the soil and may be primary or secondary pathogens of the skin and nails. Previous nail destruction by a dermatophyte, trauma, or another nail disease favors nail infection by NDMs[7]. The widespread use of broad-spectrum antibiotics, DM, corticosteroids and other immunosuppressive drugs increase the incidence of nail infection due to NDMs. Clinical differentiation between onychomycosis due to dermatophytes and NDMs is difficult as they are presented with the same clinical features[8].

Different species of NDMs as *Aspergillus* spp., *Acremonium spp*., *Fusarium spp*., *Scytalidium spp*., *Scopulariopsis spp*. and dematiaceae fungi as *Alternaria spp*., *Cladosporium* and *Curvularia* have been involved in 2-11% of onychomycosis cases[9].

Aspergillus spp. has been emerged as primary causative agents of onychomycosis worldwide. To diagnose onychomycosis due to *Aspergillus* spp., the following criteria are required: positive direct

microscopic examination and repeated culture or molecular detection of *Aspergillus* spp., provided that no dermatophyte or *Candida* spp. were isolated[10].

Clinically, onychomycosis is classified into four types; distal/lateral subungual onychomycosis (DLSO), proximal subungual onychomycosis (PSO), white superficial onychomycosis (WSO) and total dystrophic onychomycosis (TDO)[11].

The nail infection caused by NDMs is problematic not only due to the patient pain and discomfort but also due to the failure of antifungal therapy. Itraconazole and terbinafine have good *in vitro* antifungal activity against NDMs more than other antifungal agents; fluconazole and griseofulvin[12].

This study assessed the frequency of non-dermatophyte moulds causing onychomycosis among patients attending Mansoura University Hospital and their antifungal susceptibility.

METHODOLOGY

Study design:

This is a descriptive study carried out at Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, from October 2019 to March 2021. A total of 103 patients referred from Outpatient Dermatology Clinic, Mansoura University Hospital, Dakahliya Governorate, Egypt, to Mycology Lab, Microbial Diagnostic and Infection Control Unit (MDICU) at Medical Microbiology and Immunology Department, Faculty of medicine, Mansoura University were included in this study. Demographic and clinical data were collected from each patient. Patients receiving antifungal drugs due to other skin diseases were excluded from the study. This study was approved by Mansoura Faculty of Medicine Ethical Committee (R.21.05.1323) and informed written consent was obtained from each patient or their relatives.

Samples collection:

Patients' toes and fingers nails were examined for any nail abnormalities. After cleaning the nail with 70% ethanol to remove any debris, bacterial contaminant or ointments, a nail scraping was collected from the proximal edge of the diseased nail and from the junctions between healthy and diseased nails with a sterile scalpel blade in a clean dry paper envelope[13].

Samples processing:

Standard mycological tests were performed using potassium hydroxide (KOH 20%) preparation for any fungal elements, and then each sample was cultured on two plates of Sabouraud's dextrose agar (SDA) (Oxoid, UK) with and without cyclohexamide (0.5%) (Oxoid, UK) and with chloramphenicol (50µg/ml). Cultures were incubated aerobically at 25˚C-30˚C up to 4 weeks. Fungal growth was inspected daily for the first week then twice weekly for the next 3 weeks[14].

Mycological identification was established macroscopic and microscopic using Lactophenol Cotton Blue (LPCB) preparation. Slide culture on SDA was used to identify *Aspergillus* spp. and other filamentous fungi. *Candida* spp. were further identified by Gram stain, LPCB preparation, germ tube test and subculture on CHROMagar Candida plates (BD, Hungary, Germany)[15].

In vitro antifungal sensitivity testing of NDMs fungal isolates was performed by the agar disc diffusion method using five antifungal agents (itraconazole 10 µg, terbinafine 25µg, ketoconazole 10µg, fluconazole 25µg and amphotericin B 20µg (HiMedia Laboratories, Mumbai, India) according to Clinical and Laboratory Standards Institute (CLSI) M51-A method[16]. Inhibition zone of the used antifungal agents is described in table 1.

Table 1: Inhibition zone of the tested antifungal agents against NDMs by the agar disc diffusion method

Antifungal	Disc content (µg)	Zone diameter in mm		
		S	I	R
Itraconazole	10	≥17	14-16	≤13
Terbinafine	25	≥20	12-19	≤11
Fluconazole	25	≥17	14-16	≤13
Ketoconazole	10	≥18	14-17	≤13
Amphotericin B	10	≥15	10-14	≤9

Where; S: Sensitive, I: Intermediate, R: Resistant

Statistical Analysis

Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 25 (SPSS Inc, Chicago, IL, USA). Categorical variables were described as numbers and percentages. *P*-value < 0.05 indicated a significant difference.

RESULTS

This study was conducted over 17 months on 103 patients with clinically suspected onychomycosis; 37 (35.9 %) were males while 66 (64.1 %) were females. Females were the most significantly presented group with nail abnormalities and housewives were the most common occupation to be affected. Most of cases were from rural areas 74 (71.8%) (table 2).

Patient's ages ranged from 8-65 years and the mean age of the studied population was 35.7±13.9 years. The middle age groups (21-40 and 41-60) were the most significantly affected age groups with onychomycosis (P < 0.001, table 3).

Table 2: Demographic profile of the studied patients with clinically suspected onychomycosis

Variables	No.	%
Sex:		
Males	37	35.9
Females	66	64.1
Residence:		
Urban	29	28.2
Rural	74	71.8
Occupation:		
Housewives	49	47.6
Government employees	11	10.8
Manual workers	10	9.7
Farmers	9	8.7
Students	6	5.8
Laboratory technician	3	2.9
Drivers	2	1.9
Others	13	12.6
Total	103	100%

Table 3: Age distribution among the studied cases with clinically suspected onychomycosis

Age range in years	No.	%	Mean ± SD	*P value*	χ^2
<10	3	2.9	35.7± 13.9	<0.001	38.02
10-20	3	2.9			
21-40	66	64.1			
41-60	24	23.3			
>60	7	6.8			
Total	103	100%			

Where; SD: Standard deviation

The toenails affection was the most common; 72 patients (69.9%), however 28 patients (27.2%) showed fingernails affection and only 3 patients (2.9%) showed abnormalities in both fingernails and toenails. The most common presented clinical type was DLSO 68 (66.0%), followed by TDO 30 (29.1%), while both WSO and PSO were the least common presented clinical types; 4 (3.9%) and 1(1.0%) respectively. Nine patients (8.7%) had DM, 6 patients (5.8%) suffered from anemia, 3 patients (2.9%) received immunosuppressive drugs, 5 patients (4.9%) had other co-morbid disease other than nail infection and only 2 patients (1.9%) exposed to nail trauma (table 4).

Table 4: Characteristic features and risk factors for the studied patients with clinically suspected onychomycosis

Characteristics	No.	%
Site of onychomycosis:		
Fingernail	28	27.2
Toenail	72	69.9
Both	3	2.9
Clinical type of onychomycosis:		
Distal/lateral subungual onychomycosis	68	66.0
Total dystrophic onychomycosis	30	29.1
Proximal subungual onychomycosis	1	1.0
White superficial onychomycosis	4	3.9
Risk factors:		
Trauma	2	1.9
Diabetes Mellitus	9	8.7
Anemia	6	5.8
Immunosuppression	3	2.9
Pregnancy	1	1.0
Other co-morbid disease	5	4.9
No risk factors	77	74.8
Total	103	100

A total 86 fungal isolates were recovered from 106 nail specimens taken from 103 patients shared in this study. Out of these, 7 isolates (6.6 %) were dermatophytes, and 17 isolates (16.0%) were *Candida* spp.; 11 (10.4%) were *Candida albicans* and 6 (5.7%) were non-albicans *Candida*. However, NDMs were the most prevalent fungal isolates; 62 (58.5%).

Identification of the recovered NDMs revealed a significant predominance of *Aspergillus* isolates where *A. fumigatus* was the most predominant 27 (25.5%), followed by *A. niger* 19 (17.9%) and *A. flavus* 4 (3.8%). Three isolates (2.8%) of *Penicillium* spp. and *Alternaria alternata* were isolated, while 2 isolates (1.9%) of *Fusarium* spp. and *Scedosporium* spp. were isolated. Only one isolate (0.9%) of *A. terreus* and *Onychocola Canadensis* were isolated (figure 1). Clinical presentation, macroscopic culture and microscopic characters of some NDMs isolated from clinical cases of onychomycosis were illustrated in figures 2,3, 4, 5 and 6.

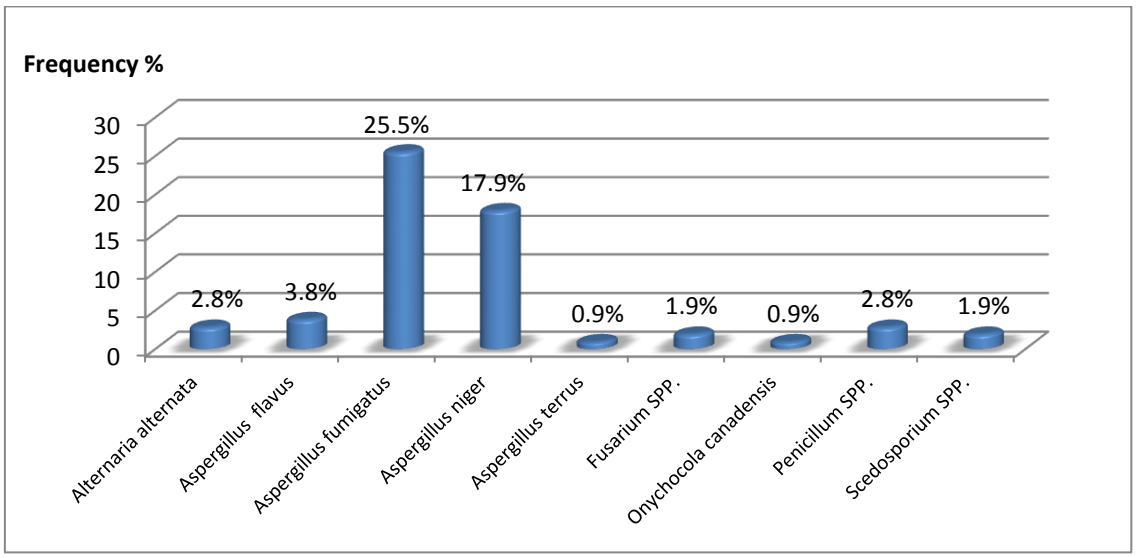

Fig.1: Frequency of non-dermatophyte moulds (NDMs) species causing onychomycosis.

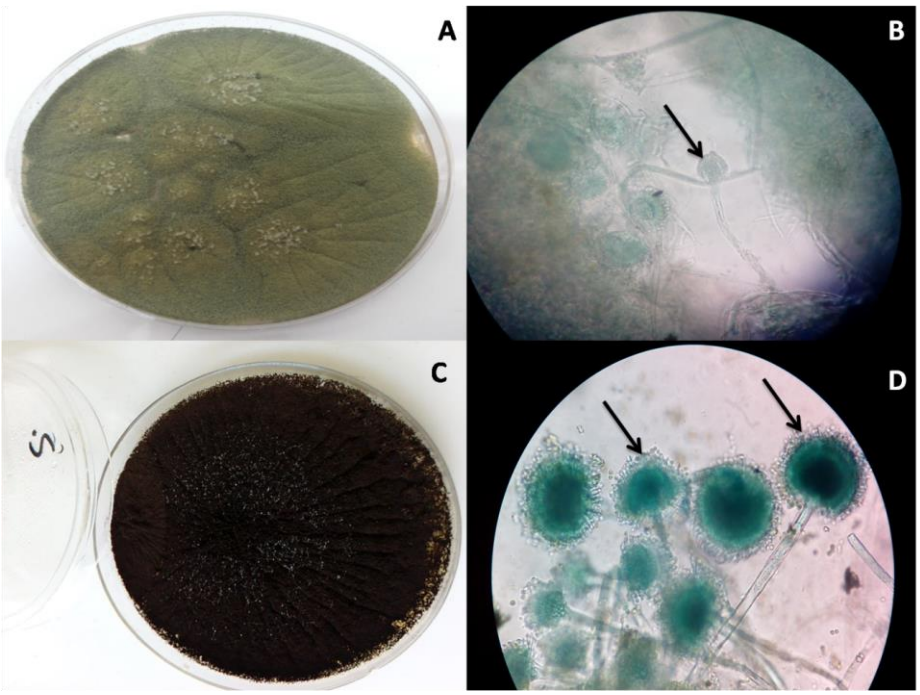

Fig. 2: A, C. Culture of *A. fumigatus* and *A. niger* on SDA, respectively. B, D. Microscopic picture of *A. fumigatus* and *A. niger* with Lactophenol Cotton Blue preparation, respectively by X40 Magnification.

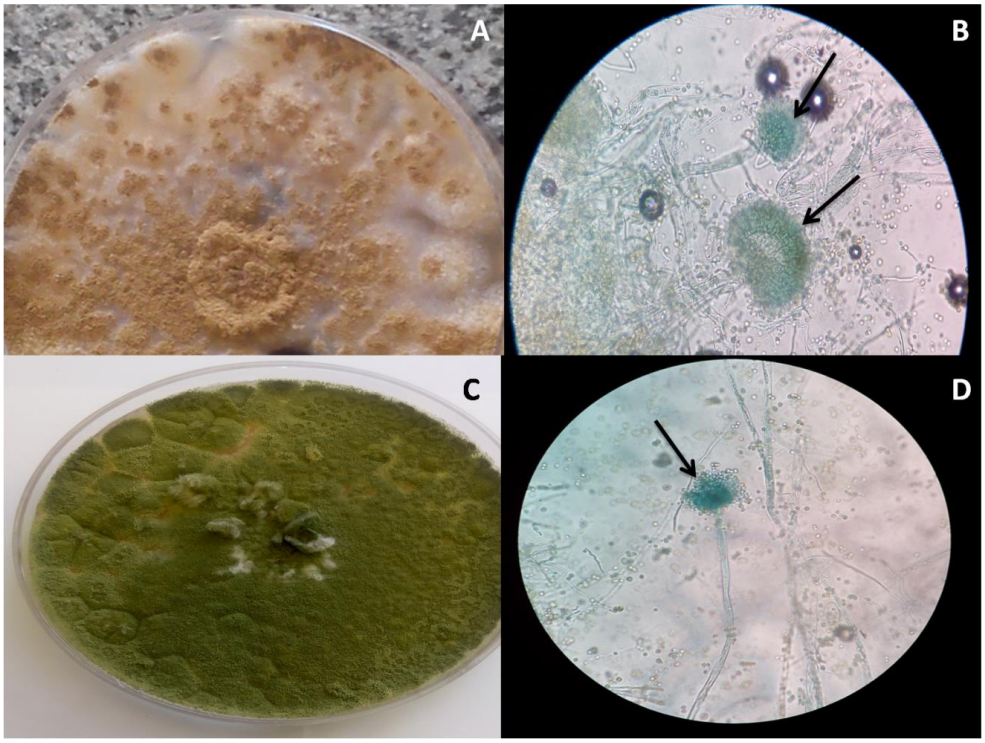

Fig. 3: A, C. Gross picture of *A. terreus* and *A. flavus* culture on SDA, respectively. B, D. Microscopic picture *A. terreus* and *A. flavus* by Lactophenol Cotton Blue stain, respectively under X40 Magnification.

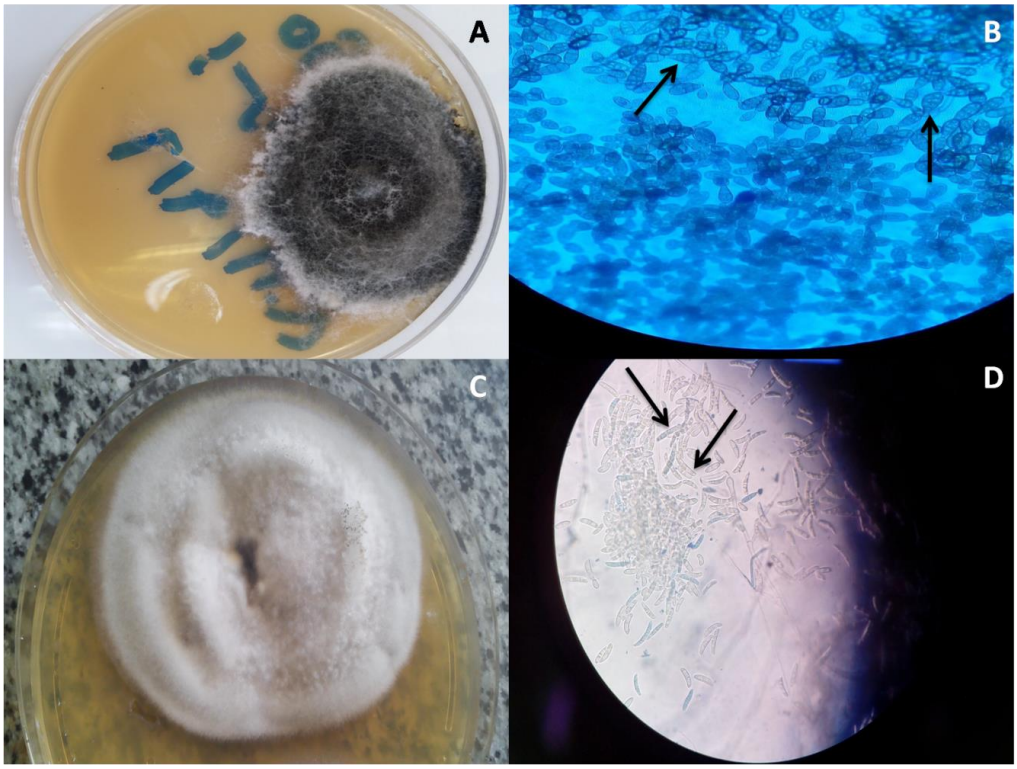

Fig. 4: A, C. Gross colony of *Alternaria alternate* and *Fusarium* spp. on SDA, respectively. B, D. Macroconidia of *Alternaria alternate* and *Fusarium* spp., respectively by Lactophenol Cotton Blue stain by X40 Magnification.

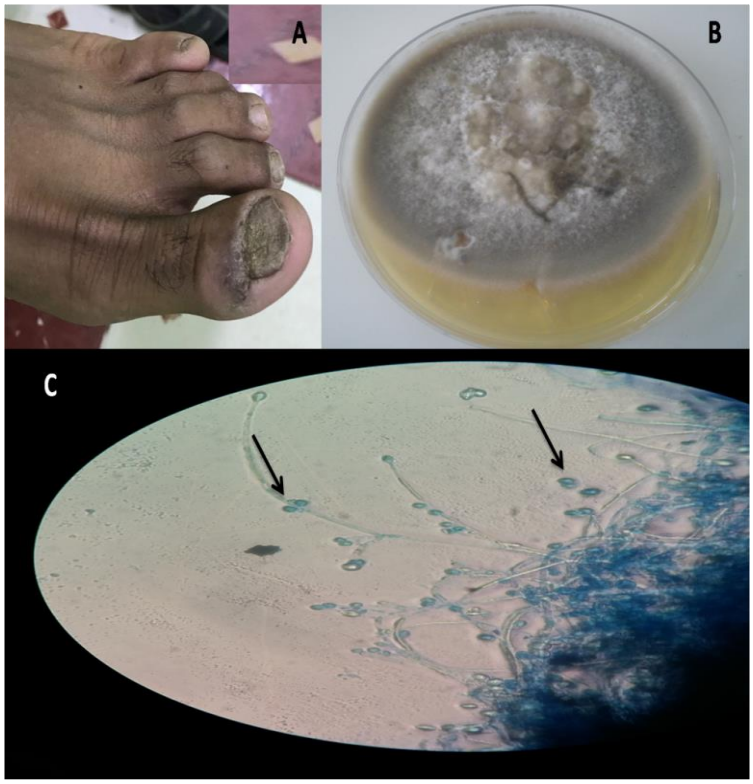

Fig. 5: A Case of toenail onychomycosis caused by *Scedosporium* species. B. Culture of *Scedosporium* spp. on SDA. C. Microscopic picture of *Scedosporium* spp. by Lactophenol Cotton Blue stain under X40 Magnification.

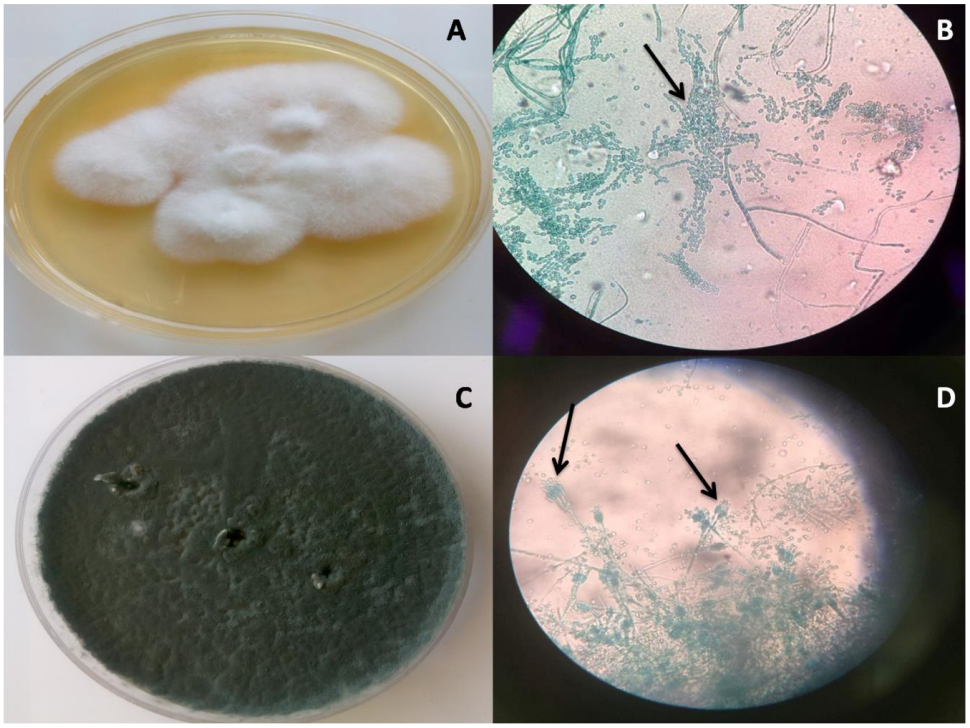

Fig. 6: A, C. Gross colony of *Onychocola Canadensis* and *Penicillium* spp. on SDA, respectively. B, D. Microscopic picture of *Onychocola Canadensis* and *Penicillium* spp., respectively by Lactophenol Cotton Blue stain under X40 Magnification.

In vitro antifungal susceptibility testing of NDMs isolates showed the high sensitivity of *A. fumigatus, A. niger, A. flavus, A. terreus, Penicillium* spp., *Alternaria alternate, Fusarium* spp., *Onychocola canadensis* and *Scedosporium* spp. to itraconazole (88.9%, 100%, 75%, 100%, 100%, 100%, 100%, 100% and 100%), respectively. The sensitivity of *A. fumigatus, A. niger, A. flavus, A. terreus, Penicillium* spp., *Alternaria alternate, Fusarium* spp., *Onychocola canadensis* and *Scedosporium* spp. to ketoconazole was (96.3%, 84.2%, 75%, 100%, 100%, 100%, 50%, 100% and 100%), respectively, while the sensitivity to terbinafine in *A. fumigatus, A. niger, A. flavus, A. terreus, Penicillium* spp. and *Alternaria alternate* was (81.5%, 68.4%, 50%. 100%, 33.3% and 100%), respectively. All *Fusarium* spp., *Onychocola canadensis* and *Scedosporium* spp. were resistant to terbinafine. All NDMs isolates showed complete resistance to fluconazole and amphotericin B (table 5).

Table 5: Antifungal susceptibility testing of NDMs isolated from patients with clinically suspected onychomycosis.

NDMs (n=62)		Antifungal agents N (%)				
		Itraconazole	**Terbinafine**	**Fluconazole**	**Ketoconazole**	**Amphotericin B**
A. fumigatus (n=27)	**S**	24 (88.9)	22 (81.5)	0 (0)	26 (96.3)	0 (0)
	I	2 (7.4)	0 (0)	0 (0)	0 (0)	0 (0)
	R	1 (3.7)	5 (18.5)	27 (100)	1 (3.7)	27 (100)
A. niger (n=19)	**S**	19 (100)	13 (68.4)	0 (0)	16 (84.2)	0 (0)
	I	0 (0)	4 (21.1)	0 (0)	3 (15.8)	0 (0)
	R	0 (0)	2 (10.5)	19 (100)	0 (0)	19 (100)
A. flavus (n=4)	**S**	3 (75)	2 (50)	0 (0)	3 (75)	0 (0)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	1 (25)	2 (50)	4 (100)	1 (25)	4 (100)
A. terreus (n=1)	**S**	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)
Penicillium (n=3)	**S**	3 (100)	1 (33.3)	0 (0)	3 (100)	0 (0)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	2 (66.7)	3 (100)	0 (0)	3 (100)
Alternaria alternate (n=3)	**S**	3 (100)	3 (100)	0 (0)	3 (100)	0 (0)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	3 (100)	0 (0)	3 (100)
Fusarium (n=2)	**S**	2 (100)	0 (0)	0 (0)	1 (50)	0 (0)
	I	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
	R	0 (0)	2 (100)	2 (100)	0 (0)	2 (100)
Onychocola Canadensis (n=1)	**S**	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)
Scedosporium (n=2)	**S**	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	2 (100)	2 (100)	0 (0)	2 (100)

Where; S: Sensitive, I: Intermediate, R: Resistant

DISCUSSION

Onychomycosis is the most common nail disorder. It accounts for up to 50% of nail diseases. Although dermatophyte and *Candida* infections were the most common causes of onychomycosis, nowadays there are increase in the numbers of onychomycosis cases due to NDMs worldwide[17].

In the present study, out of 106 collected nail samples, 86 (81.1%) were positive with a higher isolation rate among females (64.1%) than males (35.9%), which may be due to increased exposure of females to water and detergents in household activities. This result was agreed with previous studies[18,19]. In another study, onychomycosis was twice or three times more in male than female patients[20].

Patient's ages in the current study ranged from 8-65 years and the mean age was 35.7±13.9 years with a higher incidence rate of onychomycosis in age group between 21 and 60 years and a decreased rate in patients below 20 years and over 60 years in accordance with other studies[21,22]. The high rate of onychomycosis in adults may be due to the increased nail trauma and slow nail growth.

The majority of cases in our study were housewives (47.6%) with greater involvement of toenails (69.9%) compared to fingernails (27.2%) which might be due to more traumas in the nails because of occlusive foot wears. This is in accordance with some reports from Greece[23] and USA[24] and in contrary to another study from Canada which had reported that the nail infection is more in fingernails than toenails in patients working in wet environment and frequently exposed to chemicals and trauma[25].

The most common clinical presentation of onychomycosis in this study was DLSO (66.0%), followed by TDO (29.1%) in agreement with other studies[26,27].

Among 106 nail samples subjected to different mycological examinations including the direct microscopic examination using KOH 20% and culture on SDA, 81.1% revealed positive fungal growth where NDMs were the commonest cause of onychomycosis (58.5%), followed by *Candida* spp. (16.0%) and dermatophytes (6.6%) in accordance with other literatures[28,29]; however, this was on contrary to a previous study that revealed dermatophytes and *Candida* as the commonest fungal isolates in nail infection[30].

Among NDMs isolates, *A. fumigatus* was the commonest fungal isolates (25.5%) followed by *A. niger* (17.9%), *A. flavus* (3.8%), *Alternaria alternate* (2.8%), *Penicillium* spp. (2.8%), *Fusarium* spp. (1.9%), *Scedosporium* spp. (1.9%), *A. terreus* (0.9%) and *Onychocola canadensis* (0.9%). These results were comparable with another study[31], while in another study from Egypt, it has been reported that *A. niger* is the commonest fungal isolates from nail disorder[32].

The causative agents of onychomycosis differ with different geographical regions due to the variation in climatic conditions. In Sri Lanka, *Alternaria alternata* was the most prevalent fungal isolate causing onychomycosis followed by *Cladosporium* spp.[33], while in Turkey, *Fusarium* spp., *Acremonium* spp., and *Scopulariopsis* spp. were the commonest NDMs causing onychomycosis[34]. *Scopulariopsis* spp. were the predominant isolates causing onychomycosis followed by *A.terreus*, *A. niger* and *A. flavus* in another report from Iran[35].

In the present study, NDMs isolates showed a high *in vitro* sensitivity to itraconazole and ketoconazole and complete resistance to fluconazole and amphotricin B. *Aspergillus* spp. and *Alternaria alternate* showed a high *in vitro* susceptibility to terbinafine while *Penicillium* spp. showed low sensitivity to terbinafine, while *Fusarium* spp., *Scedosporium* spp., and *Onychocola Canadensis* were completely resistant to it. These results were in agreement with other published literatures[36,37]. However, in some studies, terbinafine showed a high *in vitro* sensitivity to filamentous moulds causing nail infection[38,39]. Another study has showed the insensitivity of *Fusarium* spp., to itraconazole and terbinafine and the high *in vitro* efficacy of topical amphotericin B[40].

CONCLUSION

Onychomycosis is a common nail disorder, although it is not considered a life-threatening disease, it can be a source of pain and discomfort for the patient. Non-dermatophyte moulds are nowadays a common cause of fungal nail infection and should be suspected particularly in patients with culture negative for dermatophytes or those with antifungal therapy failure. Non-dermatophyte moulds are resistant to conventional methods of treatment. Azole antifungals as itraconazole, ketoconazole and terbinafine are considered nowadays the first line of treatment of onychomycosis caused by non-dermatophyte moulds.

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- Each author listed in the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article has not been published anywhere and is not currently under consideration by another journal or a publisher.

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