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Case Report

Onychomycosis caused by a rare fungal pathogen: A neoscytalidium novaehollandiae case

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Abstract

This study presents a rare case of onychomycosis caused by non-dermatophytic fungi. A 43-year-old male patient visited the dermatology OPD with complaints of color change of his right fingernails after injury during farming work. The patient's complaints did not resolve even after one month of treatment with oral itraconazole in a private health setup. So, for further investigation, nail clipping was sent to the Microbiology laboratory at our hospital. Macroscopically, there was a total dystrophy of the nail. It was discolored. Direct microscopic examination of nail scraping with 40% potassium hydroxide, under low light at 40x, revealed septate hyphae. The nail clipping sample was cultured on SDA agar and incubated at 25°C and at 37°C. After 3 days of incubation fungal colony appeared. Macroscopically, the fungus was greenish with a powdery consistency, with no reverse pigmentation. Microscopically, phaeoid hyphae were seen with chains of arthroconidia. Based on laboratory evidence, the isolated fungus was diagnosed to be a black fungus, *Neoscytalidium novaehollandiae*. However, due to some limitations, no further investigation and molecular identification had been done. The patient was provided OPD based treatment with oral terbinafine and ciclopirox nail lacquer. The patient responded to the treatment within 3 months.

Keywords: Onychomycosis, Neoscytalidium novaehollandiae, Terbinafine.

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1. Introduction

Onychomycosis is fungal infection of nails caused by dermatophytes, non-dermatophytes or saprophytic molds, and yeast. Onychomycosis caused by dermatophytes is known as tinea unguium. 1,2 Onychomycosis leads to nail discoloration, thickening and if remain untreated may lead to severe complications such as cellulitis, osteomyelitis and nail loss.^{2,3} Neoscytalidium spp. are dematiaceous fungi leading to superficial fungal infections and rarely associated with deep cutaneous and disseminated infections mainly in immunocompromised patients.^{3,4} These are phytopathogens which can infect humans via direct contact or through traumatic wound. Neoscytalidium includes spp. dimidiatum. Neoscytalidium Neoscytalidium novaehollandiae, Neoscytalidium orchidacearum, Neoscytalidium oculi and N. hyalinum.⁵ This report details a case of onychomycosis caused by rare ascomycetous fungi *Neoscytalidium novaehollandiae*.

2. Case Presentation

2.1. Chief complaint

A 43-year-old male patient presented to the dermatology outpatient department at a tertiary care hospital, with the complaints of colour changes on the right-hand on the second finger nail for the last 3 years. The patient had no history of recent trauma on the affected finger. The patient had no significant history of diabetes or immunocompromised.

On clinical examination, brownish discoloration with depressed and ill-defined margins was noted on the patient's fingernail. It extended from the proximal nail fold to the lateral nail fold, including the cuticles. The surrounding skin

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was intact, and no changes were observed. There was no discharge or redness.

Patient was treated with oral itraconazole 200mg twice daily for one month in a private health setup but there were no signs of improvement observed with the treatment so patient was referred to tertiary care hospital.

2.2. Sample collection

The clipped nail specimen was collected with all aseptic precaution in sterile container and sent to the microbiology laboratory for further fungal culture to identify the causative organism and to treat the patient accordingly.

2.3. Laboratory diagnosis

2.3.1. KOH preparation

The specimen was treated with 40% KOH and kept for 3 hours to dissolve the specimen and then KOH mount was prepared and viewed under microscope. In KOH preparation septate phaeoid hyphae were observed. (**Figure 3**)

2.3.2. Fungal culture

Following the standard laboratory procedure, the specimen was immediately inoculated on two Sabouraud Dextrose Agar (SDA) plates without chloramphenicol and cycloheximide. The plates were then incubated at 37°C and 25°C. The SDA plates were monitored on daily basis for colony growth and identification of fungal pathogen.

2.4. Macroscopic examination

The colonies on SDA were fast-growing and appeared within 3 days of incubation at room temperature. The colony was initially white, turning greenish and brown within a week, exhibiting suppressed, fluffy, and powdery mycelium with cottony smooth edges. No colony was seen in a plate that was kept at 37°C incubation.(**Figure 1**)

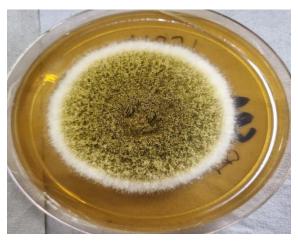


Figure 1: Colony morphology- fungal colony was greenish brown with a powdery consistency, with no reverse pigmentation

2.5. Microscopic examination (LPCB Mount)

LPCB mount: In LPCB mount phaeoid septate hyphae were seen with chains of arthroconidia. Arthroconidia were initially hyaline and aseptate, oval in shape becoming light brown in colour with maturity. (**Figure 2**)

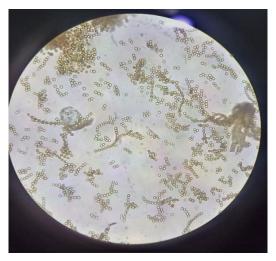


Figure 2: Chains of arthroconidia under KOH mount

2.6. Antifungal susceptibility testing

Antifungal susceptibility testing was not performed. The antifungal treatment was guided relying on established susceptibility data.



Figure 3: KOH mount of nail scraping

2.7. Treatment

The patient was provided OPD based treatment with oral terbinafine (250mg) once daily for 6 weeks and topical ciclopirox nail lacquer for 8 weeks. The patient responded to the treatment and completely recovered within 3 months. The duration of treatment will vary depending on severity and location of infection for example for toenail infections oral terbinafine is recommended for 12 weeks which is longer compared to fingernail infections.^{7,8} The molecular identification of such fungus and antifungal susceptibility testing could further help in better management of such cases.

3. Discussion

A large number of non-dermatophytic fungi are involved in skin and nail infections such as *Aspergillus flavus, Fusarium oxysporum, Acremonium species* and *Candida albicans*.^{1,2} *Neoscytalidium* species have emerged as significant cause of Onychomycosis. ^{1,9} In our study we represent a rare case of onychomycosis caused by fungus *Neoscytalidium novaehollandiae*.

The Fungus *Neoscytalidium novahollandiae* is an endophytic pathogen first isolated from a baobab tree by Pavlic *et al.* back in 2008.⁵ There was a first case report of human finger nail onychomycosis by *N. novaehollandiae* in an Iranian female in 2019 which has been mentioned in a study of Shokoohi *et al.*⁶

Among Neoscytalidium species, most common species associated with Onychomycosis are Neoscytalidium dimidiatum and Neoscytalidium hyalinum.³ Neoscytalidium novahollandiae differs from Neoscytalidium dimidiatum macroscopically and microscopically as shown in **Table** 1.^{5,6,12,13}

 Table 1: Differences in morphological appearance

Morphology Neoscytalidium Neoscytalidium		
Morphology	dimidiatum	novahollandiae

Macroscopic	Effuse, hairy or	Colonies were
appearance	woolly, dark-	initially white and
	greyish to	became greenish
	brownish-black	olivaceous within 7
	pigmented colonies	days.
	on SDA agar.	
	Grows rapidly	
	within 3 days.	
Microscopic	Conidia are	The fungus forms
appearance	ellipsoidal or round	white to olivaceous
	shaped with	aerial mycelium
	acutely rounded	containing chains
	apex and truncate	of arthroconidia.
	base initially	Arthroconidia are
	hyaline and	initially hyaline
	aseptate becoming	and aseptate, thick-
	brown and bi-	walled, oval to
	septate on	cylindrical
	maturity.	becoming one
	Branched, septate,	septate of brown
	brown hyphae are	on maturity.
	seen	Sometimes
		characteristic
		muriform conidia
		are also seen.
		Branched, septate,
		brown hyphae are
		seen

Thus onychomycosis caused by *Neoscytalidium novahollandiae* is rare but must be included in differential diagnosis. For more accurate diagnosis a comprehensive approach including KOH preparation, LPCB mount, fungal culture and molecular identification along with antifungal susceptibility testing is required.

Our patient was initially treated with oral itraconazole for one month but no improvement was observed. And then treatment with oral terbinafine was started and patient recovered.

The in-vitro antifungal susceptibility testing can be performed by broth microdilution method using Clinical and Laboratory Standards Institute (CLSI) M38-Ed3 guidelines. Very limited data is available regarding standardized and effective treatment for fungal pathogens. Thus antifungal susceptibility testing will guide therapeutic strategies and help to trace emerging resistant pattern among such phaeoid fungus. ^{13,14} Additionally newer antifungal agents must be explored for resistant and recurrent fungal infections.

4. Limitations

 Lack of molecular methods for species confirmation: No molecular techniques, such as PCR, were available at our institute for confirmation of fungal species. These molecular methods could have increased accuracy in diagnosis and help differentiate *Neoscytalidium novaehollandiae* from other ascomycetous fungi.

- Unavailability of Antifungal susceptibility testing methods: Antifungal susceptibility testing was not performed, these could provide guidance and ensure effective antifungal treatment of the patient.
- 3. Limitation of single case report: This study is single case report, thus more studies are required for better understanding regarding epidemiology, prevalence and clinical importance of non dermatophytic dematiaceous fungus as causative agent of Onychomycosis. Larger research studies will help in guiding antifungal agents for better treatment of such patients.
- 4. Lack of proper follow up: Long term follow up of patient was not done so could not trace recurrence rate.

5. Conclusion

This report highlights a rare case of non-dermatophytic phaeoid fungus as causative agent of Onychomycosis. As per morphological features seen under the LPCB mount and gross appearance of the colony, the isolated fungus was identified as *Neoscytalidium novahollandiae*. The patient completely recovered within 3 months of treatment with oral terbinafine (250mg) once daily for 6 weeks and topical ciclopirox nail lacquer.

6. Ethical Approval

This study was approved by Institutional ethical approval committee with ref. no. SV/EC/ON/Medi/RP/1904.

7. Source of Funding

None.

8. Conflict of Interest

None.

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