A dematiaceous fungus of *Neoscytalidium dimidiatum* as an emerging cause of superficial black onychomycosis: a case report

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ABSTRACT

Onychomycosis is one of the most common nail disorders. *Neoscytalidium dimidiatum* has been recognized among the common cause of onychomycosis that clinically similar to dermatophyte molds. To our knowledge, there are no reports of onychomycosis due to *N. dimidiatum* from Indonesia. Here, we describe two cases of onychomycosis, affecting females, 70 and 12 years of age. We discuss macroscopic and microscopic identification as well as the choices of antifungal therapy. The presented cases highlight that *N. dimidiatum* cannot be underestimated as the cause of onychomycosis in tropical and subtropical countries, especially if there were characteristic clinical conditions of dark-pigmented discolorization of the affected nails.

Keywords

Neoscytalidium dimidiatum; onychomycosis; case report

INTRODUCTION

Onychomycosis refers to a nail disorder caused by fungal agents. It is one of the most common nail disorders requiring visits to clinicians, with a prevalence of about 50% of all onychopathies[1]. It was estimated that globally, the prevalence of onychomycosis was 5.5%[1]. There are several risk factors for onychomycosis, including advanced age (elderly), trauma, diabetes, immunosuppresions (including HIV), and cancers[2]. Onychomycosis can be caused by dermatophyte molds (*Trychophyton* spp., *Microsporum* spp., and *Epidemophyton* spp.), non-dermatophyte molds [NDM] (*Fusarium* spp. and *Aspergillus* spp.), as well as yeast (*Candida* spp.)[3].

The fungus *Neoscytalidium dimidiatum* was previously recognized as an uncommon NDM-causing onychomycosis[4]. *N. dimidiatum* belongs to dematiaceous fungus within the *Botryosphaeriaceae* family[5]. *N. dimidiatum* is originally known as plant pathogen[6] and is currently considered as one of the emerging fungal pathogens infecting humans[7]. The taxonomic and nomenclature of *N. dimidiatum* have been constantly revised because of the presence of both hyaline and phaeoid (dark) colonies as well as the production of both arthroconidia and pycnidial synanamorphs. The previous names include *Nattrassia mangiferae,*

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Hendersonula toruloidea, Fusicoccum dimidiatum, and *Scytalidium dimidiatum*[8]. Currently, *N. oculus, N. orchidacearum,* and *N. novaehollandiae* have also been identified within the *Neoscytalidium* genus. *N. orchidacearum* and *N. novaehollandiae* are first known as plant pathogens. However, *N. novaehollandiae* is recently reported in onychomycosis cases[9]. The previous distinct species of *N. hyalinum* that microscopically similar to *N. dimidiatum* but had no black pigmentation, is classified as hyaline mutant (variant) of *N. dimidiatum (N. dimidiatum var. hyalinum)* based on molecular analysis[10, 11].

In addition to superficial infections, there are several reports describing invasive infections due to *N. dimidiatum,* including cerebrospinal fluid (CSF) invasion[12], brain abscess[13, 14], and pulmonary infections[15]. In these cases, the infected patients had underlying clinical conditions that compromised their immune functions, including post-transplant patients receiving immunosuppressive therapy (such as prednisone or anti-thymocyte globulin) and malignancy. *N. dimidiatum* has also been reported to be involved in allergic fungal rhinosinusitis[16, 17].

Onychomycosis due to *N. dimidiatum* is particularly prevalent in tropical regions. However, the disease is frequently overlooked since its clinical presentation resembles those caused by dermatophyte fungi. Notably, the treatment is challenging since it is commonly resistant to common antifungals used in the clinic[8]. In addition, to our limited knowledge, there are no reports of onychomycosis due to this fungal pathogen in Indonesia. Thus, here we report the identification of *N. dimidiatum* from two onychomycosis cases in our hospital to improve our awareness and understanding of the diseases and the pathogen itself. We discuss the macroscopic and microscopic identification as well as the choices of antifungal therapy.

CASE PRESENTATION

Case 1

The first patient was a woman, farmer, 70 years old of age presented with dark-brown discolorization of the right and left toenails **(Figure 1A)**. Fragments of the affected nails were aseptically collected due to a suspicion of onychomycosis, and they were subsequently transported to our laboratory for mycological identification. After five days of incubation on Sabouraud Dextrose Agar (SDA) plates, two distinct colonies were observed. The first colony was white and smooth. Microscopic examination with lactophenol cotton blue (LPCB) staining showed yeast. Subculture to the CHROMagar™ resulted in green colonies of *Candida albicans. In vitro* antifungal susceptibility testing was performed using the microbroth dilution method. The *C. albicans* isolate was resistant to ketoconazole, itraconazole, fluconazole, terbinafine, and micafungin.

The second colony was white and floccose on the surface, and yellow on the reserve. Microscopic examination with LPCB staining showed branched, septate hyphae without any specific morphological characteristic. To further identify the isolate, we subcultured on the SDA tube as well as performed slide culture on this isolate. On the SDA tube, the colony was initially fluffy and white on the surface and yellowish on the reverse within 2 days **(Figure 2A)**. Subsequently, it turned dark grey on the surface and black on the reverse after 8 days **(Figure 2B)**. Microscopic examination demonstrated thick-walled, dark-brown arthroconidia in chains. The arthroconidia were rectangular or barrel-shaped **(Figure 2C)**. Numerous hyaline and septated hyphae were also observed **(Figure 2D)**. The macroscopic and microscopic appearance were characteristics of a dematiaceous fungus, *Neoscytalidium dimidiatum. In vitro* antifungal susceptibility testing using the microbroth dilution method showed that the isolate was resistant to ketoconazole and itraconazole, but sensitive to fluconazole and terbinafine. There was no growth of the colony on SDA supplemented with cycloheximide. In addition, no dermatophyte fungi were identified.

Case 2

The second patient was a female, a student of junior high school, 12 years old presented with black, vertical discolorization of the fingernails **(Figure 1B)**. We received fragments of the affected nails in our laboratory to identify the fungal pathogen. After four days of incubation on SDA plates, a white and smooth colony was observed. Microscopic examination with LPCB staining revealed yeast. Subsequent subculture to the CHROMagar™ resulted in cream to white colonies of *Candida glabrata. In vitro* antifungal

susceptibility testing using the microbroth dilution method demonstrated that isolate was resistant to ketoconazole and itraconazole, but sensitive to fluconazole, terbinafine, and micafungin.

On the SDA tube, a colony of white and floccose on the surface, and yellow with a black pigment on the reserve was observed on day 4. We subsequently subcultured the isolate on an SDA plate. On the SDA plate, a wool-like and white colony on the surface and a black pigment on the reverse were already observed on day 3 **(Figure 3A)**. The plate was fully covered with dark grey on the surface and black pigmented wool-like colonies on the reverse plate on day 9 **(Figure 3B)**. To further identify the isolate, we performed slide culture on this isolate. A similar macroscopic and microscopic observation with the *N. dimidatum isolate* of case 1 was observed on the isolate **(Figure 3C)**. *In vitro* antifungal susceptibility testing using the microbroth dilution method showed that the isolate was resistant to ketoconazole, itraconazole, and terbinafine, but sensitive to fluconazole. Similar to the first isolate, there was no growth of the *N. dimidiatum* on SDA supplemented with cycloheximide. Additionally, no dermatophyte fungi were identified.

DISCUSSION

N. dimidiatum has been recognized as one of the non-dermatophyte molds (NDM) causing onychomycosis. A number of case reports can be found in the literature demonstrating the identification of *N. dimidiatum* in onychomycosis[18-22]. It has also been detected as a rare cause of cutaneous phaeohyphomycosis[23, 24]. The incidence of onychomycosis due to *N. dimidiatum* varies based on geographical regions. Among 52 cases of mycologically confirmed onychomycosis in Cameroon, *N. dimidiatum* was only identified in two cases affecting toenail, while *T. rubrum* was identified as the most common cause[25]. However, another study in French Guiana found that *N. dimidiatum* was not a rare cause of onychomycosis. *N. dimidiatum* [n=29 (24.8%)] was the second most common cause of toenail onychomycosis after *T. rubrum* [n=35 (29.9%)][26]. In contrast, *N. dimidiatum* was the leading cause of tinea pedis and onychomycosis in Thailand, followed by *T. rubrum*[27]. In a systematic review to identify the prevalence of NDM in onychomycosis, *S. dimidiatum* and *N. mangiferae* (both are the previous nomenclature of *N. dimidiatum)* were collectively identified in 27 of 156 cases (17.3%)[28]. Thus, at least in certain (tropical) regions, *N. dimidiatum* can be considered as a common

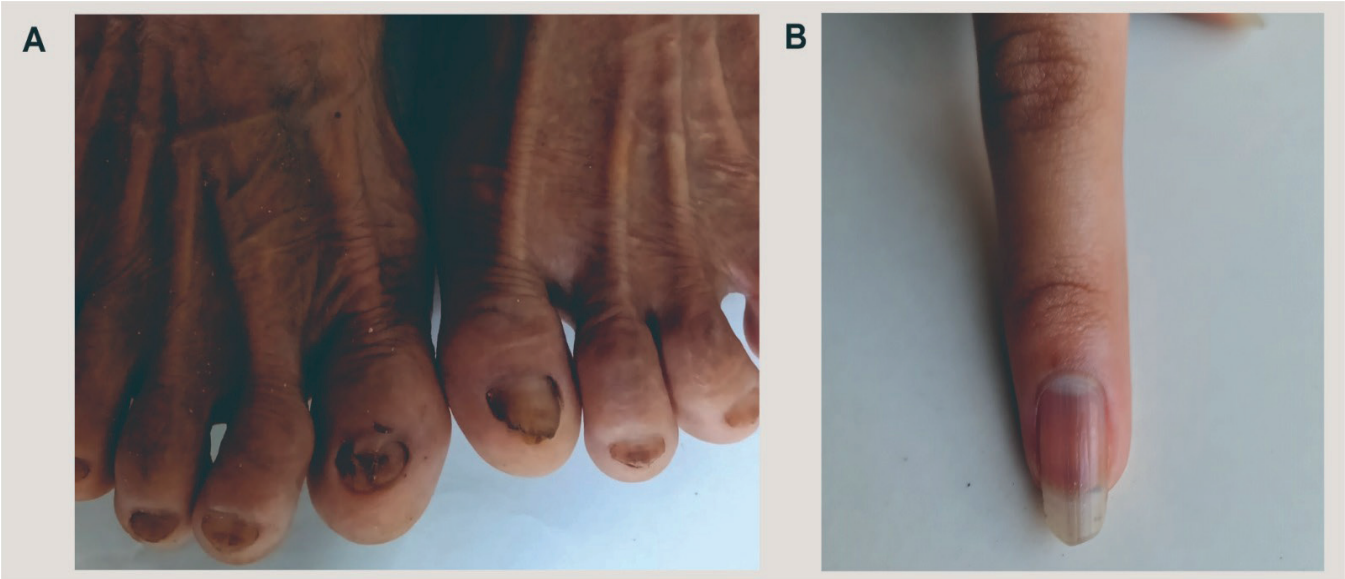

Figure 1. A. The dark brown to dark pigmented nail lesions in patient 1. **B.** The black pigmented nail lesions in patient 2.

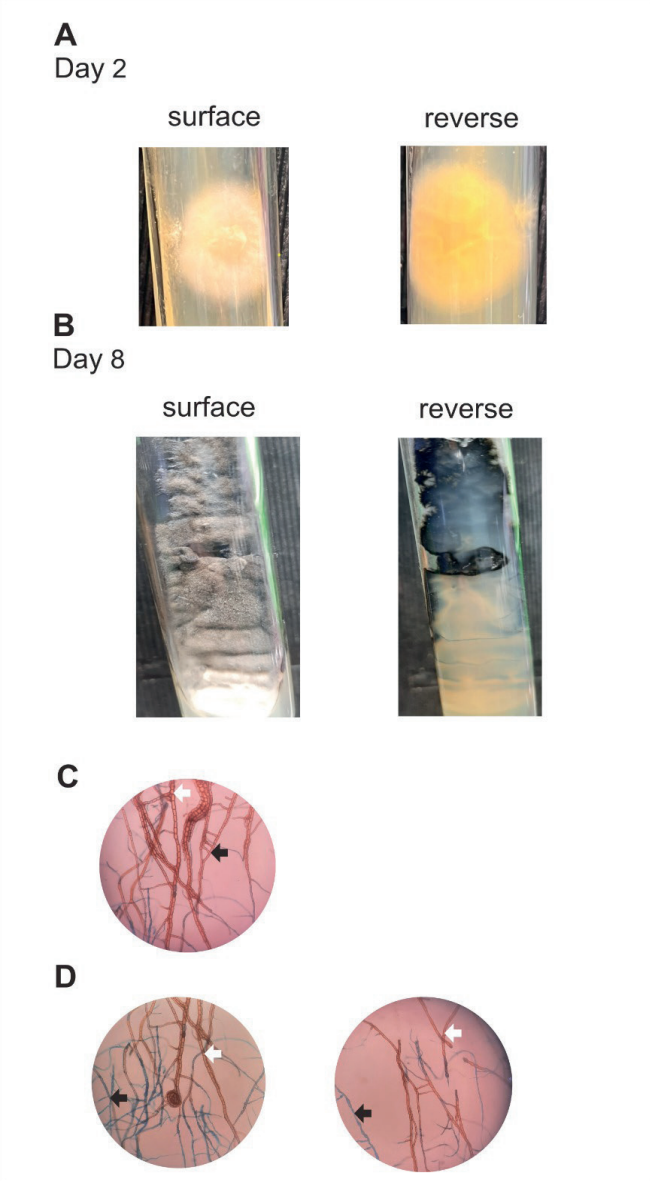

Figure 2. The macroscopic and microscopic appearance of *N. dimidiatum* isolated from patient 1. **(A)** On day 2, the colony was fluffy and white on the surface and yellowish on the reverse. **(B)** On day 8, it turned dark grey on the surface and black on the reverse plate**. (C)** Microscopic examination demonstrated thick-walled, dark-brown arthroconidia in chains. The arthroconidia were rectangular (black arrow) or barrel-shaped (white arrow). **(D)** Numerous hyaline and septated hyphae were also observed (black arrow) in addition to dark-brown pigmented hyphae (white arrow).

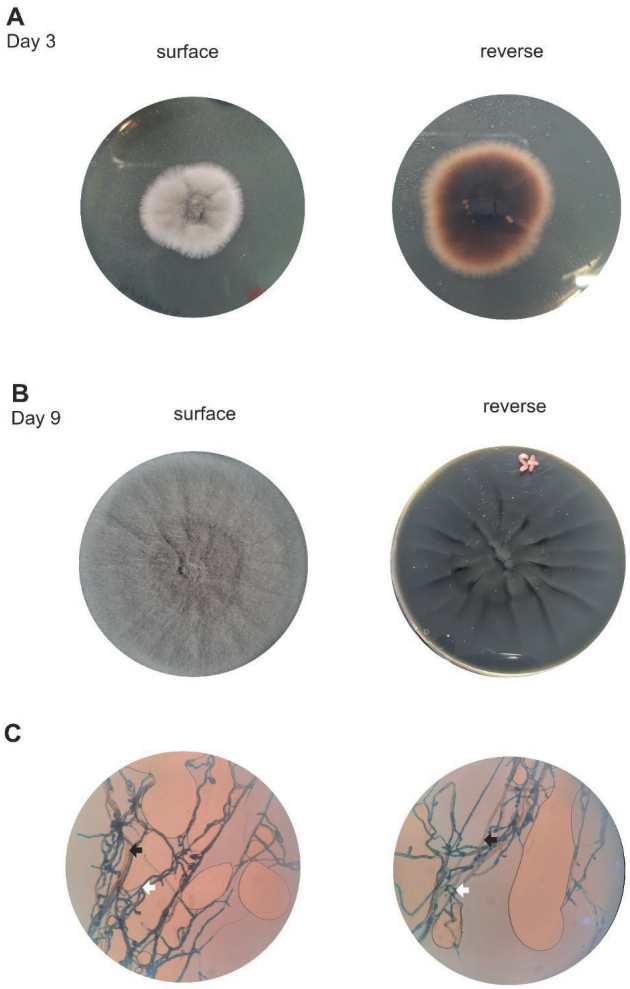

Figure 3. The macroscopic and microscopic appearance of *N. dimidiatum* isolated from patient 2. **(A)** A wool-like and white colony on the surface and a black-pigmented colony on the reverse were observed on day 3. **(B)** The SDA plate was fully covered with dark grey on the surface and black pigmented wool-like colonies on the reverse plate on day 9. **(C)** Numerous hyaline and septated hyphae were observed (white arrow) in addition to dark-brown pigmented hyphae (black arrow).

cause of onychomycosis.

In both cases, *Candida* sp. was also identified along with *N. dimidiatum.* The interpretation of fungal culture is challenging since we need to differentiate between contamination, normal flora, and true pathogen causing the diseases[29]. Since both fungal pathogens are known to cause onychomycosis[3], it is possible that both pathogens were involved in disease pathogenesis, although in these cases, the presenting clinical presentation (black discolorization of the affected nails) highly indicated that *N. dimidiatum* was predominantly involved.

The pathogenesis of *N. dimidiatum* to cause nail and skin infections was associated with its capacity to produce keratinases[30]. *N. dimidiatum* more commonly infects the nails of the feet than that of the hand, as observed in the first case who is a farmer[8]. In the second case, fingernails were affected. Although previous studies on onychomycosis due to *N. dimidiatum* showed that it mainly affected aged population of more than 50 years old[31, 32], here we found in a children patient of 12 years of age. Clinically, the nail lesion is typically characterized by black pigmentation (fungal melanonychia) due to melanin production[3, 4, 18], as present in the case 2. However, a cross-sectional study of onychomycosis caused by *T. rubrum* (n=55) and *N. dimidiatum* (n=34) from 2016-2018 revealed that the clinical presentations of both infections were similar[32]. Thus, a correct identification of the causative fungal pathogen is essential to provide adequate antifungal therapy since *N. dimidiatum* is considered as recalcitrant onychomycosis[33].

The diagnosis of onychomycosis was conventionally based on the macroscopic and microscopic observation of fungal culture[34]. *N. dimidiatum* is sensitive to cycloheximide[22], as described in our cases that they did not grow in SDA with cycloheximide supplementation. In the culture media, white and fluffy colonies are first observed within a few (3-5) days. However, they gradually become dark-grey on the surface with black on the reverse side within 7-10 days. Finally, the plate is completely covered by the growth of black wool-like colonies[5, 18]. In the microscopic examination, LPCB staining shows septate and branched hyphae. The hyphae are pigmented and thick-walled. Another characteristic is abundant dark brown-pigmented arthroconidia in chains or isolated (disarticulated). The arthroconidia could be unicellular or bicellular[5, 18, 19]. However, we did not identify bicellular arthroconidia in our present cases. *N. novaehollandiae* was recently reported to cause onychomycosis, and it was morphologically similar to *N. dimidiatum*[9]. However, *N. novaehollandiae* produces muriform, dichomera-like conidia, which differentiate this species from *N dimidiatum* and other species within *Neoscytalidium* genus[35].

Species-level identification based on macroscopic and microscopic examination of cultures is hampered because of intraspecies morphological pleomorphism[36]. Accurate identification of fungal pathogens to genus and species level is essential for epidemiological viewpoint, as well as for precise antifungal therapy[34]. Molecular identification is achieved by sequencing of internal transcribed spacer (ITS) region of ribosomal DNA (rDNA)[37]. Sequencing analysis of ITS rDNA region is required to differentiate *N. dimidiatum* from other species within *Neoscytalidium* genus, such as *N. novaehollandiae*[9]. In addition, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) is a mass spectrometry that is increasingly used in clinical microbiology laboratory for organism identification. Several studies have shown its good performance for fast identification of *Neoscytalidium* sp.[37, 38]. However, MALDI-TOF still needs a viable pure culture for identification, so it can not be directly employed from a clinical sample[34].

Currently, there is no standardized treatment for onychomycosis due to *N. dimidiatum.* Antifungal choices can be guided by *in vitro* susceptibility testing against common antifungals. Antifungal susceptibility test of *N. dimidiatum* clinical isolates in Malaysia showed that amphotericin B, voriconazole, miconazole, and clotrimazole had high inhibitory activities against these isolates with minimum inhibitory concentration (MIC) ranging from 0.0313 to 1 μg/mL, while most isolates had high MICs (>16 μg/mL) for itraconazole, ketoconazole, and fluconazole[39]. Similar findings were reported from two *N. dimidiatum* clinical isolates in Japan[19]. Another study evaluating *in vitro* susceptibility of 30 clinical isolates of *N. dimidiatum* showed a low susceptibility to itraconazole (MIC ≥16 μg/mL) and a high susceptibility to terbinafine (MIC ≤0.25 μg/mL)[32]. High susceptibility to amphotericin B led to successful

therapy in invasive *N. dimidiatum* infections[13, 24].

In the first case, the patient initially received topical ketoconazole 2% as an empiric antifungal treatment. However, the patient did not yet attend a follow-up visit at the time of writing. Consequently, we could not change the antifungal treatment based on the antifungal susceptibility test's results. Thus, a follow-up by phone was conducted. The patient mentioned that although the nail lesions did not improve yet, she refused to continue the medication since she thought that it did not interfere with her daily activities as a farmer. In the second case, the patient received fluconazole 150 mg per oral once a week and topical ketoconazole 2% twice daily. After five months of treatment, the nail lesions were significantly improved. The treatment is still on going at the time of writing. In contrast to the first case, the patient thought that the nail lesions resulted in cosmetic problems, and therefore, she complied with the medication and follow-up schedule. These two cases highlighted the importance of education for the patients to comply with the antifungal therapy.

CONCLUSION

In conclusion, we report two case reports of onychomycosis due to *N. dimidiatum* from our hospital. To our best knowledge, this is the first report of onychomycosis caused by *N. dimidiatum* in Indonesia. We discuss the macroscopic and microscopic identification as well as the choices of antifungal therapy based on *in vitro* antifungal susceptibility testing. Importantly, this report highlights the importance of *N. dimidiatum* to be included in the differential diagnosis of onychomycosis since it clinically resembles dermatophytes and is a common disease in tropical and subtropical countries.

AUTHORS CONTRIBUTION

Data gathering and idea owner of this study: M. S. H., T. W.

Study design: M. S. H., T. W.

Data gathering: M. S. H., N. R., H. R.

Writing and submitting manuscript: M. S. H.

Editing and approval of final draft: M. S. H., N. R., H. R., T. W.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL CLEARENCE

Our institution does not require ethical approval for reporting case report or case series.

INFORMED CONSENT

Written informed consent was obtained from the patient or the legally authorized representative for information to be published in this article.

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