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Brief Report

Brief Report

Talaromyces atroroseus in HIV and non-HIV patient: A first report from Indonesia

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Abstract

We performed morphology, molecular study and antifungal susceptibility test on 10 *Talaromyces* sp. isolates: eight clinical isolates (human immunodeficiency virus (HIV) and non-HIV-patient) and two isolates from rats. All strains produced red soluble pigment and microscopically showed *Penicillium*-like structure in room temperature and yeast-like structure in 37°C. Based on molecular analysis, nine isolates were identified as *Talaromyces atroroseus* (including the isolates from rats) and one as *T. marneffei*. Our susceptibility result of *T. marneffei* supports the use of amphotericin B, itraconazole for talaromycosis marneffei management. *Talaromyces atroroseus* showed variable MIC to echinocandin, azole derivatives, 5-flucytosine and amphotericin B.

Key words: *Talaromyces atroroseus*, *Talaromyces marneffei*, Indonesia, ITS, *BenA*.

Some species in genus *Talaromyces* can cause talaromycosis. *Talaromyces marneffei* is the most well-known species causing talaromycosis.[1] Besides *T. marneffei*, *Talaromyces* other than *marneffei* had been isolated from clinical specimen.[2–7] After the arrival of the acquired immune deficiency syndrome (AIDS) pandemic, cases increased sharply in Thailand, Vietnam, China, Malaysia, and other Asian countries.[8–11] However, data on talaromycosis in Indonesia are scarce, probably due to lack of alertness and standard diagnostic laboratory procedures for fungal disease in Indonesia. We did morphology, molecular study and antifungal susceptibility test on 10 suspected *Talaromyces* sp. isolates (eight isolates from patient material and two from rats).

This study included 10 isolates from three human immunodeficiency virus (HIV) patients, four non-HIV patients, one patient who had unknown clinical status, and two isolates from rats caught in the house of a patient (TM1-TM10). All isolates were phenotypically identified as *T. marneffei* and deposited in the culture collection of Mycology Laboratory, Department of Parasitology, Faculty of Medicine, Universitas Indonesia. Morphology study was conducted on malt extract agar (MEA-Oxoid, UK) at 25°C and on brain heart infusion (BHI-Liofilchem, Italy) agar at 37°C for 10 days. Amplification of the internal transcribed spacer (ITS) region (primers ITS1 and ITS4) and part of beta tubulin (*BenA*) gene (primers Bt2a and Bt2b) using polymerase chain reaction (PCR) was performed according to Visagie et al.[12] The generated sequences were deposited in GenBank (ITS, Accesion number MK396611-MK396620; *BenA*, Accesion number MK448217-MK448226). A homology search was performed using BLAST analysis in GenBank and the ITS database

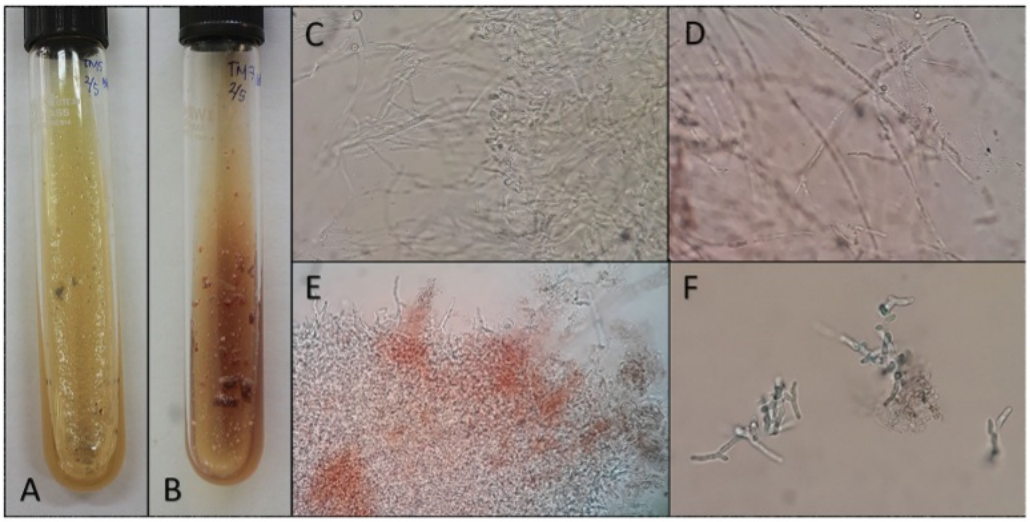

Figure 1. Morphology of the isolates at 37°C (10 days). Colony on brain heart infusion (BHI) agar representing isolates TM1-6 and TM8-10 (A); colony on BHI representing isolates TM7 (B); microscopic appearance of isolate TM5, representative for isolates TM1-6 and TM8-10 (C & D); microscopic appearance of isolate TM7 (E & F). This Figure is reproduced in color in the online version of *Medical Mycology*.

of International Society for Human and Animal Mycology (ISHAM). Phylogenetic trees were constructed using the MEGA v.6.0.6 software. Maximum likelihood was used to analyze the aligned ITS and *BenA* data sets, and the statistical support of the phylogram was determined using 1000 bootstraps. Tamura 3-parameter +G+I substitution model was used for the ITS data set and Kimura 2-parameter +G substitution model was used for the *BenA* data set.[1] Susceptibility study was conducted using modified Sensititre™ YeastOne YO10 (Thermo Scientific, UK) for *Aspergillus*. In brief, inoculum was 5 days old culture in 37°C instead of 35°C.[13] Ethical approval was obtained from the Health Research Ethic Committee, Faculty of Medicine, Universitas Indonesia (Ref. Num. 304UN2.F1/ETIK/2017).

On MEA at 25°C, TM1-6 and TM8-10 had a similar macromorphology with *T. atroroseus* and TM7 with *T. marneffei*, and both produced red soluble pigment and showed *Penicillium*-like structure. The result was similar to study of Yilmaz et al.[1] At 37°C on BHI agar, both species showed a different micromorphology (Fig. 1). After 10 days, TM7 grew as a yeast-like colony producing dark red pigment diffusing into the medium, while the colonies of TM1-6 and TM8-10 were more filamentous and did not produce soluble pigments (Fig. 1A and 1B). Microscopically, TM7 produced arthrospores (Fig. 1F), short hyphae (4.42–4.92 μm wide), and some cells resembling yeast (diameter 5.74–6.30 μm) (Fig. 1E). The hyphae of TM1-6 and TM8-10 appeared to be fragmented (arthrospore?) and longer (±3.98 μm wide) (Fig. 1D). Cells resembling yeast were also observed (diameter 4.74–5.57 μm) (Fig. 1C). Moreover, biopsy with giemsa staining in two of our HIV patients with skin lesion and cervical lymph node ulcer (TM3 and TM4) showed yeast cells inside phagocyte appearance resembling *T. marneffei* [R. Wahyuningsih, data not shown].

Most isolates (TM1-6, TM8-10) were identified as *T. atroroseus* based on molecular study. With exception of one (TM10), all *T. atroroseus* isolates had identical ITS sequences. The *BenA* sequences were identical for all *T. atroroseus* isolates. This sequence data indicate that these isolates are similar but other typing techniques (e.g., MLST) are needed to confirm this. The generated phylogenetic trees (data not shown) showed *T. atroroseus* belongs to section *Trachyspermus*, while *T. marneffei* belongs to section *Talaromyces*. Our *T. atroroseus* isolates clustered together with isolates CBS 257.37 (air, Germany), CBS 234.60 (unknown source, Germany), CBS 133447 (cheese warehouse, The Netherlands), CBS 133449 (mouse dung, Denmark) and CBS 133450 (soil, Australia), showing the worldwide distribution of this species. We also isolated *T. atroroseus* (TM1 and TM2) from *Rattus rattus* caught in the house of patient TM3. The isolation of *T. atroroseus* from rat's lung and liver was the first in the world and could indicate animal as reservoir and possibility of zoonotic transmission of this fungus. *Rattus rattus* had never been a reported reservoir of *T. atroroseus*. We also tried to isolate the fungus from soils outside patient TM3's house but failed despite repeated attempts. Such difficulties were also experienced in *T. marneffei* isolation attempts from soil.[14–16]

Two isolates (TM3 and TM4) were isolated from HIV-infected patient. We confirmed *T. atroroseus* was the cause of infection since microscopically intracellular yeast was found and confirmed by culture. While in non-HIV-infected patient, the fungus was isolated from sputum, stool and nasopharyngx. Guevara-suarez et al. also found *Talaromyces* other than

Table 1. Result of antifungal susceptibility test using Sensititre™ YeastOne.

Antifungal agents	Minimum inhibitory concentration (MIC)									
	T. marneffei				*T. atroroseus*					Range
	TM7	TM1&2	TM3	TM4	TM5	TM6	TM8	TM9	TM10	*T. atroroseus*
Anidulafungin	4	>8	>8	>8	>8	>8	>8	>8	>8	>8
Micafungin	>8	>8	>8	>8	>8	>8	0.12	>8	>8	0.12– >8
Caspofungin	4	>8	>8	>8	>8	>8	0.5	>8	>8	0.5– >8
5-Flucytosine	2	>64	4	8	>64	>64	0.06	32	8	0.06– >64
Posaconazole	≤0.008	0.5	0.25	0.25	>8	0.5	0.25	0.5	0.25	0.25– >8
Voriconazole	0.03	>8	4	8	>8	>8	0.12	>8	8	0.12– >8
Itraconazole	≤0.015	1	0.25	0.25	>16	0.5	0.12	0.5	0.5	0.12– >16
Fluconazole	4	>256	>256	>256	>256	>256	64	>256	>256	64– >256
Amphotericin B	1	4	2	4	>8	4	0.5	2	2	0.5– >8

T. marneffei, Talaromyces marneffei.
T. atroroseus, Talaromyces atroroseus.

marneffei in clinical samples but did not describe the origin of the samples.[5] Isolation from unsterile sites could not support *T. atroroseus* role as the cause of infection because contamination must come into consideration.

In this study, we only found one case of talaromycosis marneffei. This fungus was isolated by touch biopsy from an HIV patient with skin lesions. This is the first *T. marneffei* talaromycosis case in Jakarta. The isolate shares identical ITS and *BenA* sequences with strains isolated from Indonesia, Vietnam, China, and Thailand isolates, indicating a close relationship. Further molecular epidemiology study using techniques that have a higher resolution is needed for confirmation.

To date, standard treatment for *T. marneffei* is amphotericin B for 2 weeks, continued by itraconazole for 10 weeks.[17] We tested the strain for amphotericin B, azoles, echinocandin, and 5-flucytosine. Our result showed that *T. marneffei* is more susceptible to posaconazole, itraconazole, voriconazole, amphotericin B, 5-flucytosine, consecutively. It showed high minimum inhibitory concentration (MIC) for echinocandin (4– >8 μg/ml) and fluconazole (Table 1). Therefore, for the strain tested, we support the use of amphotericin B and itraconazole as treatment for talaromycosis marneffei.

There is no standard treatment for talaromycosis other that marneffei, including talaromycosis atroroseus. No susceptibility study has been conducted previously for *T. atroroseus*. Our study showed variable result. Echinocandin showed MIC above 8 μg/ml for all but one strain showed low MIC for micafungin and caspofungin. Posaconazole and itraconazole showed low MIC except for one isolate showed high MIC for both drugs. Voriconazole had high MIC (4– >8 μg/ml) except for one strain (0.12 μg/ml). Fluconazole was totally resistant (MIC 64– >256 μg/ml). Amphotericin B showed variable result (MIC 0.5– >8 μg/ml), while MIC of 5-flucytosine ranging from 4 to >64 μg/ml except for one isolate showed MIC 0.06 μg/ml (Table 1). These results tell us that susceptibility pattern of *T. atroroseus* is variable and strain dependent, which was similar to previous study conducted on *Talaromyces* other than *marneffei* even though species was not mentioned.[18]

In conclusion, we found *T. atroroseus* as predominant isolate in this study. However, small number of the isolate prevents us from concluding that *T. atroroseus* is the main etiological agent of talaromycosis in HIV and non-HIV patient. Active surveillance must be conducted. Treatment of *T. atroroseus* is should be based on antifungal susceptibility test.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper. No funding is provided for the study.

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