Brief Report

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

Sem Samuel Surja, Robiatul Adawiyah, Jos Houbraken, Anna Rozaliyani, Ridhawati Sjam, Evy Yunihastuti and Retno Wahyuningsih

1Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, 2Department of Parasitology, Faculty of Medicine, Universitas Indonesia, 3Westerdijk Fungal Biodiversity Institute, The Netherlands, 4Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, 5Department of Parasitology, School of Medicine and Health Sciences, Universitas Katolik Indonesia Atma Jaya, 6Rumah Sakit Dr Cipto Mangunkusumo, Department of Internal Medicine and 7Department of Parasitology, Faculty of Medicine, Universitas Kristen Indonesia

∗To whom correspondence should be addressed. Retno Wahyuningsih, Department of Parasitology, Faculty of Medicine, Universitas Indonesia. Tel: +6221 3102135; Fax: +6221 39832018; E-mail: retnet@hotmail.com

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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, Accession number MK396611-MK396620; BenA, Accession number MK448217-MK448226). A homology search was performed using BLAST analysis in GenBank and the ITS database.
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 35°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 macro-morphology 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 microscopic morphology (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 nasopharynx. Guevara-suarez et al. also found Talaromyces other than
Table 1. Result of antifungal susceptibility test using Sensititre™ YeastOne.

<table>
<thead>
<tr>
<th>Antifungal agents</th>
<th>T. marneffei (MIC range)</th>
<th>T. atroroseus (MIC range)</th>
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<tbody>
<tr>
<td></td>
<td>TM7</td>
<td>TM1&amp;2</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>4</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Micafungin</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>4</td>
<td>&gt;8</td>
</tr>
<tr>
<td>5-Flucytosine</td>
<td>2</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>≤0.008</td>
<td>0.5</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.03</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>≤0.015</td>
<td>1</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>4</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

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

...marneffei in clinical samples but did not describe the origin of the samples. 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. 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 than 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.

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.

References


