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Rodent is Potential Reservoir of Zoonoses Fungi in Jakarta, Indonesia

Sem Samuel Surja[1,3], Robiatul Adawiyah[2], Anna Rozaliyani[2], Ridhawati Sjam[2],Retno Wahyuningsih[2,4,a)]

[1]*Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia*
[2]*Department of Parasitology, Faculty of Medicine, Universitas Indonesia*
[3]*Department of Parasitology, School of Medicine and Health Sciences, Universitas Katolik Indonesia Atma Jaya*
[4]*Department of Parasitology, Faculty of Medicine, Universitas Kristen Indonesia*

[a)]Corresponding author : retnet2002@gmail.com

Abstract. Some potentially human pathogenic fungi are known to be zoonoses. Rodent is one of potential reservoir of fungi. To date, little is known about the role of rodent in transmitting fungal disease in Indonesia. Therefore, purpose of this study was to find evidence of potential fungal zoonoses in rodent. We caught two house rats, one rat was from the house of patient suffering from talaromycosis and the other one was from a healthy person house. These rats internal organs (lung, liver, and spleen) were inoculated onto sabouraud dextrose agar (SDA) and SDA with additional of chloramphenicol. Fungi grown in the medium were analyzed using polymerase chain reaction (PCR) of internal transcribed spacer (ITS) continued by sequence-based approach. In addition PCR was also conducted using primers developed from beta tubulin gene. Amplified regions were sequenced and compared to database that contains reference sequences.We found three fungal species. *Talaromyces atroroseus* was isolated from the rat that caught from the house of patient with talaromycosis, while *Purpureocillium lilacinum* and *Penicillium citrinum* were isolated from the other rat caught in the house of healthy individual. Although naturally could be found in the environment, these species had been reported to cause fatal systemic mycosis in human. In conclusion *Talaromyces atroroseus, Purpureocillium lilacinum* and *P. citrinum* could be found in rat. This result indicates that rat could be a reservoir for these fungi.

Keywords: rat, fungi, mycosis, zoonoses, Jakarta

INTRODUCTION

Zoonoses is any disease or infection that is naturally transmissible from vertebrate animals to humans [1]. Fungus is one of infectious agent that could be transmitted zoonotically [2-5]. Some potentially human pathogenic fungi are known to be zoonoses such as dermatophyte (from domestic mammals), *Talaromyces marneffei* (from bamboo rat and domestic animal), *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, and many more [2].

Rodent is one of potential vector for zoonotic disease [2-5]. Virus is the most common infectious agent that could be transmitted by rodent, followed by significant number of bacteria and parasite.[4] Little is known about the role of rodent for transmitting of fungal infection. Most well-known rodent-borne fungal disease is talaromycosis marneffei which was third most common opportunistic infection HIV patient in Thailand [6]. In Indonesia, we rarely find zoonotic fungal infection research especially related to rodent-borne disease.

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Therefore, purpose of this study was to find evidence of potential fungal zoonoses in rodent. Finding transmission mode of disease will affect prevention of the disease.

METHODS

Samples

We caught two rats, one rat was from the house of HIV infected patient suffering from talaromycosis and the other was from healthy person house. All rats were caught by mouse trap, anesthesized using ether then sacrificed. Rats were cleaned by using 96% alcohol and dissected by using sterile scalpel. Lungs, livers, and spleens were removed and were inoculated in sabouraud dextrose agar (SDA) (Oxoid, Hampshire, England) and SDA with additional of chloramphenicol (Indofarma, Jakarta, Indonesia) to inhibit the grow of bacteria. Culture was incubated at room temperature for 10 days. Colony was observed and described. Microscopic morphology was examined by using lactophenol cotton blue (LPCB) stain [7].

Polymerase Chain Reaction (PCR)

Fungi grown in the medium were analyzed with PCR using primers developed from internal transcribed spacer (ITS) continued by sequence-based approach. In addition PCR was also conducted using primers developed from beta tubulin gene. Two step extraction method using phenol-chloroform-isoamylalcohol was used for fungal deoxyribonucleic acid (DNA) extraction [R. Wahyuningsih, unpublished results]. The DNA obtained was measured by Nanodrop (Varioskan Flash™). PCR of ITS was performed by adding 2 µL of fungal DNA master mix that contain 5 µL 10x NH_4 reaction buffer, 2 µL $MgCl_2$ 50 mM, 1 µL dNTP 10 mM, 1 µL forward primer 10 pmol, 1 µL reverse primer, 1 µL BIOTAQ™ DNA polymerase (Bioline), and 37 µL ddH_2O. Forward and reverse primer used for ITS region were ITS1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS4 (5-TCCTCCGCTTATTGATATGC-3), while primers for beta tubulin gene were bt2a (5-GGTAACCAAATCGGTGCTGCTTTC-3) bt2b (5-ACCCTCAGTGTAGTGACCCTTGGC-3) [8]. Polymerase chain reaction were conditioned as follow: pre-denaturation 95^{o}C (5 minutes), followed by 40 cycles of denaturation 95^{o}C (30 seconds), *annealing* 55^{o}C (30 seconds), extension 72^{o}C (30 seconds), and ended by final extension 72^{o}C (7 minutes).

Data analysis

Amplicon was sequenced with Sanger sequencing (both forward and reverse method). DNA sequence was then compared to National Center for Biotechnology Information (NCBI) database for both ITS region and beta tubulin gene and International Society for Human and Animal Mycology (ISHAM) ITS database for ITS region to obtain precise fungal species identification.

RESULTS

Culture

We found three different fungal culture from two rats. One species (R1) was isolated from the rat was caught from the house of patient suffering from talaromycosis and two other species (R2 and R3) from the other rat. R1 and R3 had identical fungal colony except R1 produced red pigment that diffuse to surrounding medium while R3 didn't. These three species had the same fungal structure microscopically (Fig 1). Based on this observation, we conclude that there were three different Talaromyces species. Identification by molecular method was done to this three species. Full description of the culture was written in Table 1.

Sequence analysis

Analysis using ITS region and part of beta tubulin gene gave consistent results on comparison to both NCBI and ISHAM ITS. We found three different species: R1 was *Talaromyces atroroseus*, R2 was *Purpureocillium lilacinum*, and R3 was *Penicillium citrinum*.

TABLE 1. Morphology and molecular analysis of the samples

Source	Sample name	Colony	Microscopic morphology	Sequence analysis
Rat 1	R1	Dark Green velvety to powdery colony with red pigment diffusion to surrounding medium	Penicillium-like structure	*Talaromyces atroroseus*
Rat 2	R2	Red velvety to powdery colony, with no pigment production to medium	Penicillium-like structure	*Purpureocillium lilacinum*
Rat 2	R3	Dark green velvety to powdery colony, no pigment production to medium	Penicillium-like structure	*Penicillium citrinum*

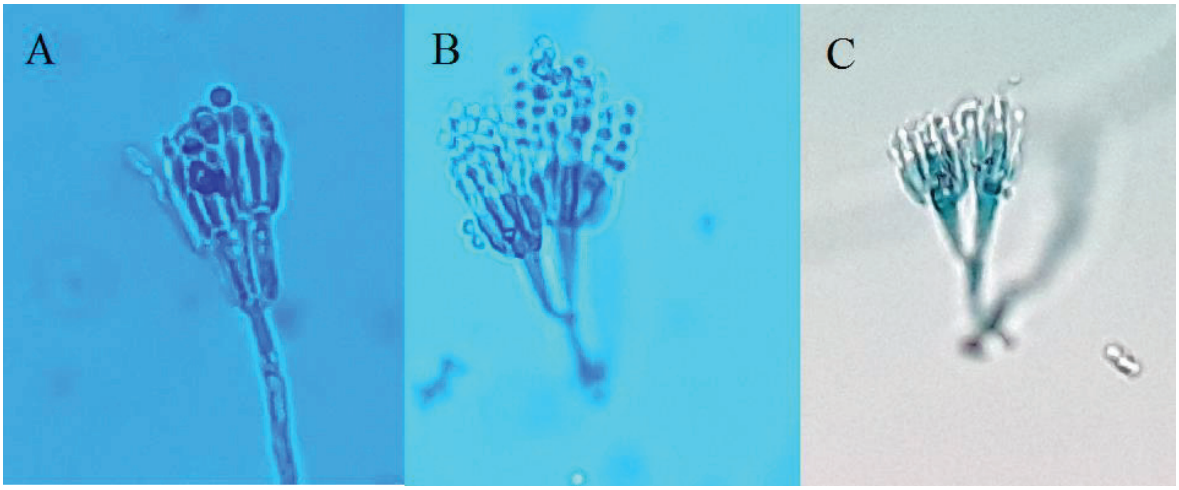

FIGURE 1. Morphology of the isolates; (A) R1; (B) R2; (C) R3. All isolates showed penicillium-like structure in LPCB stained slide (magnification 400×).

DISCUSSION

To our knowledge, no study has been conducting to find fungal isolate from rodent in Jakarta, Indonesia. We were able to isolate *T. atroroseus* from one rat. This species was once thought to be *Penicillium sp.* based on morphologic study, but species identification was then confirmed by molecular study. *Talaromyces atroroseus* was recommended to be used in food industry because its ability to produce red pigment [9]. To date, only *Talaromyces marneffei* is known to cause morbidity in human especially in immunocompromised patient [6]. Few study found *Talaromyces atroroseus* in clinical specimen, but its role on causing mycosis still debatable [10]. It is important to note that the rat was caught on patient suffering from talaromycosis' house. Based on sequence comparison, both isolates (from human and rat's isolate) had identical ITS region and part of beta tubulin sequences (published elsewhere). Isolation of this species this species in house rat internal organ and patient show the possibility of zoonotic transmission of this fungus.

We were able to isolate *Purpureocillium lilacinum* from second rat. This species is actually the same species as *Paecilomyces lilacinus* [11]. This pathogen is known as medically important fungi that can cause systemic mycosis, keratitis, and onychomycosis especially in immunocompromised patient [12-16]. Isolation of this

species from rat's internal organ may show possible zoonotic transmission of this fungus. It is important to note that this second rat was caught in healthy person's house. As far as we know, the house owner doesn't complain any illness that can be suspected as mycosis. This fact is also fit current knowledge that *P. lilacinum* was found mostly in immunocompromised patient.

Last species that we found was *Penicillium citrinum*. This species is abundant in environment and also commonly considered as laboratorium contaminant [17]. Reports of *Penicillium citrinum* infection is scarce. Despite its ubiquity, human infection is rare and mostly happened in patient who underwent immunosuppressive therapy such as chemotherapy drug or steroid. Since respiratory system is point of entry of this fungus, lung disease is the most common clinical presentation although cutaneus infection was also reported [17-20].

We suspect that all these fungi have rodent as their reservoir. Finding of few fungal species in rat should increase our awareness that rodent could potentially transmit fungal disease to human, especially when human immune system is compromised. Further research assessing more extensive fungal infection in rat should be made to confirm this early finding.

CONCLUSION

Talaromyces atroroseus, P. citrinum and P. lilacinum could be found in rat. This result indicates that rat could be a reservoir or vector for these fungi.

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