



Pulmonary Cryptococcosis due to *Cryptococcus laurentii* Co-infection with Miliary Tuberculosis in Adolescent Patient in Indonesia: Case Report

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Abstract

Background: Non-neoformans cryptococci were once believed to be harmless saprophytes with no risk to human health. *Cryptococcus laurentii* is commonly found in the environment and in pigeon feces, and has been reported as an emerging human fungal pathogen that causes infections in immunocompromised hosts. Here, we report a unique case of pulmonary cryptococcosis co-infection with Miliary Tuberculosis.

Case: A 14-year-old underweight boy was admitted to the Emergency Unit with the chief complaint of fever, cough, chest pain, breathlessness, night sweats, malaise, and weight loss. He was referred from the Public Health Centre in Sleman, Yogyakarta, with Miliary Tuberculosis and consumed six weeks of fixed-dose combinations of the first-line anti-tuberculosis drug. Microbiology culture of good quality patient's sputum revealed *Cryptococcus laurentii* along with *Klebsiella oxytoca*. The patient was diagnosed with Pulmonary Cryptococcosis. The patient's symptoms, especially fever and chest pain, did not improve with oral Fluconazole 200 mg treatment. However, he had an excellent response to Levofloxacin 500 mg during hospitalisation and oral Fluconazole therapy at 400 mg/day for eight weeks. After eight weeks of Fluconazole treatment, the patient got well, and then Fluconazole was stopped. Anti-tuberculosis treatment was administered for six months, and no recurrence was found.

Conclusion: This article reports the rare pulmonary involvement of *Cryptococcus laurentii* in adolescent patients with miliary tuberculosis in Indonesia. A high degree of pulmonary mycosis suspicion and techniques improvement for respiratory specimen collection, culture, and identification contribute to early diagnosis and fungal infection treatment. In addition, interprofessional discussion can improve patient treatment and outcomes.

Keywords: *Cryptococcus laurentii*, co-infection, fluconazole, miliary tuberculosis

1. INTRODUCTION

Cryptococcus species were previously considered environmental saprophytes, but they have now become global pathogens (1). Non-neoformans *Cryptococcus* species have been generally regarded as nonpathogenic saprophytes (2). However, in recent years, opportunistic infections associated with non-neoformans *Cryptococcus*,

including *Cryptococcus laurentii*, have been reported (3, 4). *Cryptococcus laurentii* is present in environments in droppings and cloacal samples of feral pigeons (3, 4). It has recently been reported as a cause of pulmonary, cutaneous, and any other organ infections in humans and is responsible for serious infections. We report a case of Pulmonary Cryptococcosis due to

Cryptococcus laurentii co-infection with Miliary Tuberculosis in an adolescent immunocompetent patient. The clinical resolution occurred after oral fluconazole administration and was followed by six months of Anti-tuberculosis drugs.

2. Case

A 14-year-old underweight boy (with Body Mass Index 15) was admitted to the Emergency Unit of Universitas Gadjah Mada Academic Hospital, Yogyakarta, in June 2019. The patient was referred from a Public Health Centre in Sleman, Yogyakarta. He complained of fever, cough, chest pain, breathlessness, night sweats, malaise, and weight loss. He was previously hospitalised and diagnosed with Miliary Tuberculosis two weeks ago at the private hospital, and then referred to the Pediatric Intensive Care Unit (PICU) at Universitas Gadjah Mada Academic Hospital. He had consumed rifampicin, isoniazid, ethambutol/ RHE for six weeks. He stayed in Boarding school; none of his friends had the same symptoms, but his father had a chronic cough.

Physical examination at the emergency department revealed fever (40,5 °C), with crackles and rales on auscultation of both lungs. In contrast, a chest x-ray revealed Miliary Tuberculosis, a right superior mediastinal mass, and lymphadenopathy (Figure 1). He had clubbing fingers. He was hemodynamically stable, with a systolic pressure of 120 mmHg, a diastolic pressure of 80 mmHg, a respiratory rate of 24 breaths per minute, and an oxygen saturation of 98%. The initial laboratory test revealed leucocytosis (White Blood Cell (WBC) $13,3 \times 10^3/\mu\text{l}$ (normal count was $4-11 \times 10^3/\mu\text{l}$), Neutrophils 81,2%, Lymphocyte 9,36%, Monocyte 7,26%, Basophil 0,3%, Haemoglobin was 11,5 g/dl (normal count was 12- 15 g/dl), Haematocrite was 35% (normal was 40-54%), Thrombocyte Count was $248 \times 10^3/\mu\text{l}$, Ureum was 26,9 mg/dl (normal count was 10,7-42,8), Creatinin was 0,47 mg/dl (normal count was 0,6-1,3 mg/dl), Serum Glutamic Oxaloacetic Transaminase (SGOT) 66 U/L (normal count was 20-40 U/L), Serum Glutamic Pyruvic Transaminase (SGPT) was 186 U/L (normal count was 10-40 U/L) and Albumin was 3 g/dL (normal count was 3,50- 4,8 g/dL) and Pro Calcitonin was

two ng/mL (normal count was < 0,5 ng/mL). He was rehydrated and received an Amikacin injection of 800 mg as an empiric antibiotic in the Emergency unit. This patient was then hospitalised in the negative pressure isolation room and diagnosed with Miliary Tuberculosis under six weeks of intensive phase treatment of Fixed-dose combination Anti-tuberculosis.

On the second day of hospitalisation, his hemodynamics were unstable, with his heart rate at 160 times per minute, systolic blood pressure at 90 mmHg, diastolic at 60 mmHg, respiratory rate at 35 breaths per minute, and fever at 40,5 °C. He was transferred to the Intensive Care Unit, and the antibiotic was escalated to Meropenem 1 gram per 8 hours and assessed with Sepsis. On the 5th day of hospitalisation, Multi-Slice Computed Tomography (MSCT) of the Lung was done. His MSCT of the Lung showed Tuberculosis Millier with paratracheal and right hilar lymphadenopathy. Treatment was continued with Meropenem, but he still had a fever. On the sixth day of hospitalisation, the Pulmonologist suspected that this patient probably had other fungal or bacterial infections. Sputum was obtained from the patient aseptically and inoculated onto blood agar, MacConkey agar, and Sabouraud Dextrose Agar (SDA). These agars were incubated aerobically at 37 °C. On the 8th day of hospitalisation, at SDA and blood agar, a creamy white yeast and a vast, creamy, round colony were observed as harmful gram-negative bacteria (Figure 2). The microscopic examination of the yeast revealed round yeast on India Ink, Nigrosine, and LPCP staining (Figure 3). Identification of bacteria and fungi was made with the VITEK 2 system (bioMérieux, Inc., Hazelwood, MO). Antifungal susceptibility testing for Ketoconazole, Itraconazole, Fluconazole and Terbinafine using *Candida* sp. standard for Minimum Inhibitory Concentration (MIC) was done. He was treated with Fluconazole 200 mg per 24 hours orally, then continued at 100 mg per 24 hours for the next four days.

On the 9th day of hospitalisation, Pro Calcitonin was two ng/mL. The sputum culture revealed *Klebsiella oxytoca*, susceptible to the first generation of Cephalosporin, Gentamycin, Amikacin, Amoxicillin-Clavulanic Acid, Piperacillin-

Tazobactam, Cefepime, Ceftriaxone, Levofloxacin, Meropenem, Trimethoprim-sulfamethoxazole, and Tigecycline. During the 10th hospitalisation, a fungal culture, identified as *Cryptococcus laurentii*, was obtained (Table 1). The result of the antifungal susceptibility test showed that the *Cryptococcus laurentii* isolate was sensitive to Fluconazole (Table 1). On the 11th day of hospitalisation, Levofloxacin 250 mg per 12 hours was given as a definitive antibiotic, and anti-tuberculosis treatment was continued. The thorax X-ray was re-evaluated and still showed Miliary Tuberculosis and Right Paratracheal and hilar lymphadenopathy. On the 12th day of hospitalisation, his body temperature still ranged

above the normal range, between 38 °C and 40,5 °C, under routine antipyretic treatment. Pulmonologist, Paediatrics, and Microbiologist had discussed increasing the Fluconazole dose to 400 mg per 24 hours. The patient's temperature gradually decreased after 400 mg of Fluconazole treatment (Figure 3). He was discharged on the 16th day of hospitalisation, and Fluconazole 400 mg/day was continued for eight weeks. Anti-tuberculosis treatment was administered for six months. After six months, the patient recovered well, and there was no recurrence of infection.

Table 1. Laboratory test and Culture Result during Hospitalization

Date	Test	Result
28 June 2018 (1 st day)	Laboratory Result	WBC 13,3x 10 ³ / µl, Erythrocyte 4,6 x 10 ⁶ /µl, Neutrophils 81,2%, Lymphocyte 9,36%, Monocyte 7,26%, Basophil 0,3%, Haemoglobin 11,5 g/dl, Haematocrite 35%, Thrombocyte Count 248 x 10 ³ / µl, Urea 26,9 mg/dl, Creatinine 0,47 mg/dl, SGOT 66 U/L, SGPT 186 U/L, Albumin 3 g/dL, Total bilirubin 1,6 mg/dL, Direct bilirubin 1,2 mg/dL
	Urinalysis	Cloudy color, Protein +1, Bilirubin +1, Urobilinogen +1, Blood +2, Leucocyte 1-2, Erythrocyte 20-25, Epithel 6-8, ketone negative, nitrite negative
	Chest X-Ray	Miliary tuberculosis, right superior mediastinal mass, and Lymphadenopathy
29 June 2019 (2 nd day)	Blood Culture 2 sides	Negative
	Dengue NS1 Ag	Negative
2 July 2019 (5 th day)	Laboratory Result	SGOT 34 U/L, SGPT 72 U/L, Total bilirubin 0,5 mg/dL
	Chest MSCT with contrast	Tuberculosis Miliary with paratracheal and right hilar lymphadenopathy
3 July 2019 (6 th day)	Urinalysis	Normal finding
5 July 2019 (8 th day)	Laboratory Result	WBC 6,2x 10 ³ / µl, Erythrocyte 4,0x 10 ⁶ /µl, Neutrophils 66,4%, Lymphocyte 15,9%, Monocyte 9,8%, Basophil 0,3%, Haemoglobin 10,0 g/dl, Haematocrite %, Thrombocyte Count 221 x 10 ³ / µl, SGOT 45 U/L, SGPT 41 U/L
6 July 2019 (9 th day)	Procalcitonin	2 ng/mL
	Sputum Culture Result	<i>Klebsiella oxytoca</i> , susceptible to Cephalosporin (Cefepime, Ceftriaxone), Gentamycin, Amikacin, Amoxicillin-Clavulanic Acid, Piperacillin-Tazobactam, Levofloxacin, Meropenem, Trimethoprim-sulfamethoxazole, and Tigecycline. Resistant to Ampicillin
	Chest X-ray	<i>Cryptococcus laurentii</i> , susceptible to Fluconazole, and resistant to Ketoconazole, Itraconazole, Terbinafine
		Miliary Tuberculosis and right paratracheal and hilar lymphadenopathy
9 July 2019 (12 th day)	Laboratory Result	SGOT 33 U/L, SGPT 27 U/L

12 July 2019 (16th day)
Laboratory Result

WBC $8,3 \times 10^3/\mu\text{l}$, Haemoglobin 10,6 g/dl, Haematocrite 32%,
Thrombocyte Count $430 \times 10^3/\mu\text{l}$, Erythrocyte $4,1 \times 10^6/\mu\text{l}$

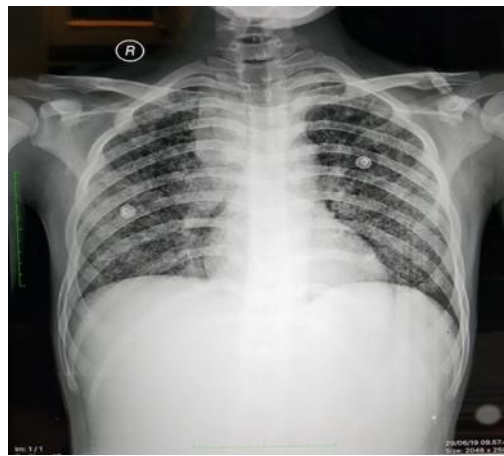


Figure 1. Chest X-ray at the Emergency Unit showed widening of the superior mediastinum to the right, and tiny needle-like opaque lesions scattered in both lungs revealed Miliary Tuberculosis. In addition, there was a right superior mediastinal mass and lymphadenopathy

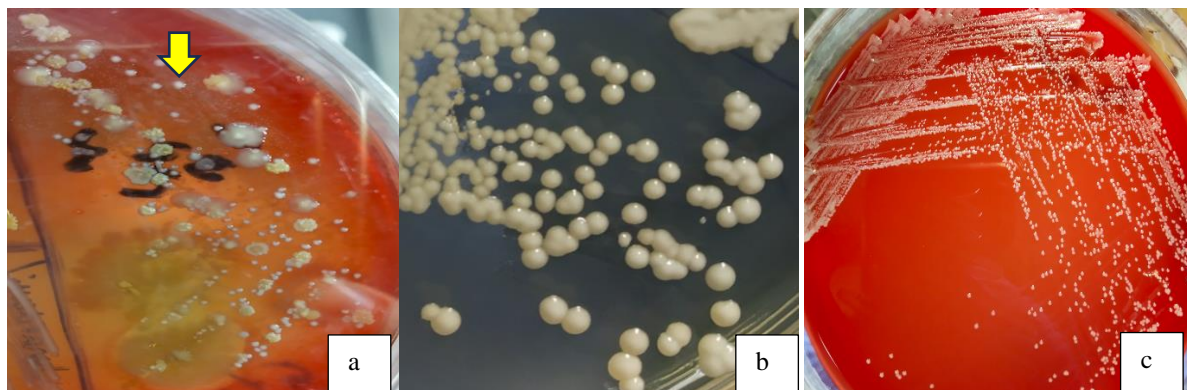


Figure 2. Bacterial and fungal cultures from the patient's culture. Blood agar showed a small white colony as yeast in microscopic (arrow), and a wide creamy colony as negative gram bacteria later recognised as *Klebsiella oxytoca* (a). *Cryptococcus laurentii* in Sabouraud Dextrose Agar showed a round, creamy white colony (b), and *Cryptococcus laurentii* in blood agar showed a small, round, white, creamy colony (c).

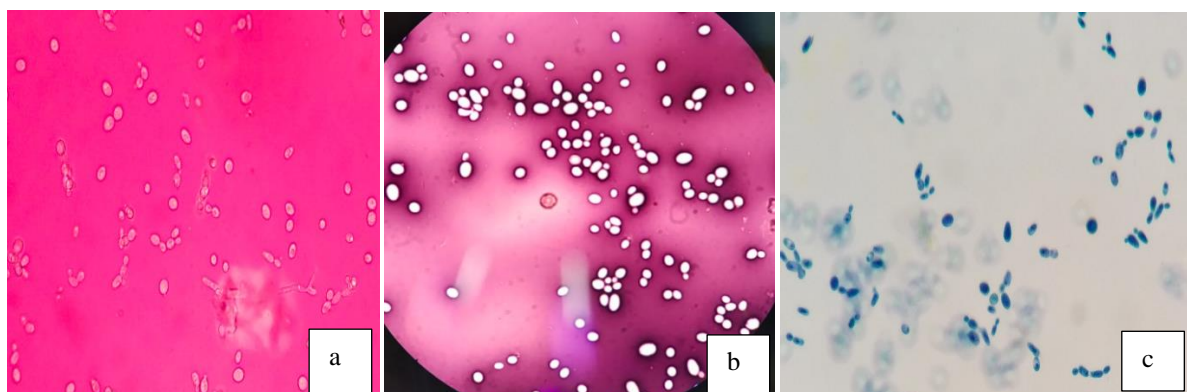


Figure 3. Microscopic appearance of *Cryptococcus laurentii* showed round yeast in India Ink staining (a), Nigrosine staining (b), and LPCB staining (c)


Clinical Sign and Symptoms	Diagnosis	Treatment and Management
1st Hospitalisation: Private Hospital referred to PICU UGM Hospital Fever, dyspneu, fatigue	14 -19 June 2019 Milliary TB, Hypoalbumin	Intensive phase anti TB drug
2nd Hospitalisation : Public Health Center in Sleman Fever, dyspneu, fatigue Refer to UGM Hospital	28 June – 13 July 2019 Milliary TB, Dehydration	Patient was referred to UGM Hospital. Rehydration& Amikacin injection 800 mg.
Emergency room: 1st day: 28 June 2019: Fever, dyspneu, chest pain, night sweat, fever (40,5 °C), crackles and rales. Heart Rate 150x/min, Respiratory rate 24x/min, Blood pressure 100/80 mmHg. PICU UGM Hospital: 2nd day: 29 June 2019 Isolation ward: 5th day: 2 July 2019 Isolation ward : 6-8th day : 3-5 July 2019	Sepsis	Blood Culture was negative. Meropenem 1 gram every 8 hours.
Isolation ward: 9th -13th day: 6-10 July 2019 Body temperature was 38 °C - 40,5 °C, under routine antipyretic treatment. Ward: 14th day: 11 July 2019 Body temperature was 38 °C - 40,5 °C, under routine antipyretic treatment. Ward: 16th day: 13 July 2019 Body temperature was 37 °C, improve appetite, nausea, dyspneu, chest pain was not found → Discharged.	Suspicious Fungal infection Milliary TB Pulmonary cryptococcosis	Sputum collection aseptically for Bacterial and Fungal Culture Meropenem 1 gram every 8 hours Empiric treatment: Fluconazole 200 mg every 24 hours orally, continued at the next days with Fluconazole 100 mg every 24 hours. Fluconazole 100 mg every 24 hours, Meropenem was stopped, Levofloxacin 250 mg every 12 hours, anti-tuberculosis treatment FDC Intensive Phase Isoniazid, Rifampicin, Pyrazinamid, Ethambutol (RHZE). The pulmonologist, pediatrician, and microbiologist of this patient discussed and decided to increase the Fluconazole dose to 400 mg every 24 hours.
Outpatient: 17 July 2019	Milliary TB Pulmonary cryptococcosis	Fluconazole 400 mg/ day, FDC Intensive Phase: RHZE, and oral Levofloxacin 500 mg/day for 14 days.
Outpatient: 31 July 2019 Patient had no complain. Fever, Cough was not found.		Fluconazole 400 mg/ day for 14 days, FDC Continuation Phase Isoniazid and Rifampicin (RH).
Outpatient: 14 August 2019		Fluconazole was stopped.
Outpatient: 6 September 2019 Outpatient 2 October 2019		FDC Continuation Phase Isoniazid and Rifampicin (RH) 5 th Month Treatment Acid Fast Smear of Sputum was Negative FDC Continuation Phase Isoniazid and Rifampicin (RH) 6 th Month Treatment
Outpatient 30 October 2019		Chest X-ray : milliary tuberculosis was improved , FDC was stopped
 <div>Cured</div>		

Figure 4. Clinical symptoms and treatments during hospitalisation and outpatient treatments

3. DISCUSSION

Cryptococcus species were previously considered environmental saprophytes, but they have now become a global pathogen (1). In Indonesia, the incidence of Cryptococcosis in AIDS was 7,540 annually (5). It was rarely pathogenic to immunocompetent humans, but recently has been reported to cause infection in many organ systems, such as the bloodstream, central nervous system, pulmonary disease, and other body sites, such as the skin, eyes, gastrointestinal tract, and lymph nodes (2).

Cryptococcus laurentii has many other names, such as *Torula laurentii* (1920), *Torulopsis laurentii* (1934), and *Cryptococcus laurentii* var. *Laurentii* (1952), *Rhodotula laurentii* (1960), *Rhodotula nitens* (1963) (6). *Cryptococcus laurentii* is commonly found in the environment and pigeon faeces and can infect humans, causing infections such as pneumonia, cutaneous abscess, peritonitis, fungemia, meningitis, and knee infection, but is mostly unrecognised by many clinicians (3, 4). Based on the integrated phylogenetic classification of the Tremellomycetes, its current name is *Papiliotrema laurentii* (6).

In patients with pulmonary Cryptococcosis, a compromised immune system is often linked to conditions such as HIV infection, diabetes, and malignancy, as well as to organ transplantation or immunosuppressive therapy (7-10). Meanwhile, Chronic *Mycobacterium tuberculosis* infection can result in anergy of the immune system to many antigens (11). One review study stated that more than 50% of pulmonary cryptococcosis patients have no risk factor or immune disorder. Approximately 60% of HIV-negative individuals diagnosed with pulmonary Cryptococcosis have no apparent underlying medical conditions (12). The increasing cases of non-neoformans cryptococci infection have been reported in immunocompromised and immunocompetent hosts (13, 14). In this case, the patient was non-HIV, with no malignancy nor diabetes mellitus, but Chronic *Mycobacterium tuberculosis* infection can probably act like a secondary immunodeficiency (11).

Fungal infection was thought to be the co-infection in this patient. Co-infection means the occurrence of at least two genetically distinct infectious agents within the same host (15). In a patient with depletion of the immunological state, like in this patient, the clinical manifestation of fungal infection was atypical (15). Because the percentage of co-infection between tuberculosis/HIV infection and fungal infection is 13,9% in atypical signs of fever of unknown origin, the diagnosis of fungal infection must be considered as co-infection, and the diagnosis of fungal infection should not be delayed or neglected (16).

Mycobacterium tuberculosis and *Cryptococcus* sp. have the capacity to cause a primary pulmonary infection and subsequently disseminate to cause severe, life-threatening systemic disease (12, 17, 18). Pulmonary Cryptococcosis and tuberculosis showed similar clinical and radiological manifestations that can delay or mask the diagnosis of the other (19). Pulmonary co-infection of *Cryptococcus* sp. and *Mycobacterium tuberculosis* occurs in both immunocompetent and immunocompromised individuals (20-23). The documentation of co-infection cases in immunologically healthy individuals indicates the possibility of mutual, yet poorly defined, predisposing conditions (20-22, 24-26).

The specimen collection, the quality of specimens, and the role of the microbiology laboratory are the key factors of fungal infection diagnosis (10, 27, 28). Lung biopsy (tissue), Broncho Alveolar Lavage (BAL), and pleural fluid are some specimens from respiratory tract infection for fungi culture. Still, only some hospitals have the capacity and facility for this invasive specimen collection (10). Histopathology examination of lung biopsy tissue can be added as an additional examination (10). In this patient, microscopic examination and fungal culture were done from good-quality sputum. Although only proven and probable pulmonary Cryptococcosis needs antifungal therapy, in this case, we present good-quality sputum that can help clinicians diagnose pulmonary Cryptococcosis and the patient was cured after appropriate treatment (12). In this case, *Cryptococcus laurentii* was

identified by the Vitek 2 System for Rapid Identification of yeasts and yeast-like organisms. An antifungal susceptibility test was done by the MIC method. The molecular examination by polymerase chain reaction (PCR) provides higher sensitivity and specificity in yeast identification (12). But *Cryptococcus* molecular testing was not done in this case because of the limitation in molecular facilities.

Cryptococcus laurentii has recently been reported to be one of the emerging human fungal pathogens with low activity to Fluconazole, susceptible to Polyenes (Amphotericin), and resistant to Echinocandin (Caspofungin) (13). Although it was known to have low activity against Fluconazole, the result of fungi culture and antifungal susceptibility test of *Cryptococcus laurentii* revealed susceptibility to Fluconazole. Therefore, this patient was treated with 200 mg/day oral Fluconazole, but the symptoms were still present, especially fever. Thus, based on guidelines, antifungal therapy for symptomatic, mild/moderate disease of the immunocompetent patient was Fluconazole 400 mg/day for six months, or Itraconazole 400 mg/ 6 months (29). Fluconazole 400 mg for eight weeks was the optimal treatment for *Cryptococcus laurentii* infection in this adolescent patient without any report of drug-induced liver injury.

In this case, the collaboration between clinicians and microbiologists successfully obtained information about other pathogens that possibly co-infect Miliary Tuberculosis and decided on the treatment. In addition, a high degree of pulmonary mycosis suspicion in Miliary Tuberculosis patients and improvement techniques for specimen collection, culture, and identification contribute to early diagnosis and treatment of fungal infection.

4. CONCLUSIONS

The lesson learned from this case is that co-infection of *Mycobacterium tuberculosis* and *Cryptococcus* can hinder the diagnosis of pulmonary Cryptococcosis. Pulmonary Cryptococcosis must be considered in a Miliary Tuberculosis patient who has not improved with anti-tuberculosis treatment or as a co-infection. It's crucial to diagnose and administer an

appropriate dose of antifungal medication in *Cryptococcus laurentii* co-infection with Miliary Tuberculosis. High suspicion, good specimen collection, and precise diagnostic tools are needed to diagnose rare fungal infections. Antifungal treatment is prescribed to the patient based on clinical diagnosis and antifungal susceptibility results.

5. ABBREVIATIONS

AIDS : Acquired Immunodeficiency Syndrome

BMI : Body Mass Index

HIV : Human Immunodeficiency Virus

MIC : Minimum Inhibitory Concentration

MSCT: Multi-Slice Computed Tomography

PCR : polymerase chain reaction

PICU: Pediatric Intensive Unit

SGOT: Serum Glutamic Oxaloacetic Transaminase

SGPT: Serum Glutamic Pyruvic Transaminase

WBC: White Blood Cell

6. ACKNOWLEDGEMENTS

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7. ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The research conducted on Pulmonary Cryptococcosis was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine-Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta (Ref. No. KE/FK/1093/EC/2022). Informed Consent to participate was obtained from the patient before the manuscript was submitted and is available from the corresponding author.

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