IDENTIFICATION OF *Aspergillus sp* IN PULMONARY TUBERCULOSIS PATIENTS WITH ADVANCED TREATMENT IN KABILA HEALTH CENTER, BONE BOLANGO REGENCY

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ABSTRACT

The purpose of this study was to determine the types of Aspergillus sp that attack pulmonary tuberculosis patients who were currently undergoing advanced treatment at the Kabila Health Center, Bone Bolango Regency. The method used in this research was a qualitative approach with a descriptive type of research.

The type of data used was primary data in the form of examination results of Aspergillus sp. at the Microbiology Laboratory of Bina Mandiri University, Gorontalo, while secondary data was in the form of observations in the form of medical records of pulmonary tuberculosis patients who undergo advanced treatment at the Kabila Health Center, Bone Bolango Regency. Sources of data were collected using questionnaires and documentation results. The sampling technique in this study used the Total Sampling technique, with a total sample of 13 people.

Based on the results of this study, from 13 sputum samples examined, it is found that 4 pure isolates of Aspergillus fumigatus are isolated.

Keywords: Aspergillus sp, Pulmonary Tuberculosis

INTRODUCTION

Infectious diseases are a serious threat in many countries because their impact can hinder the development of a country. One of the infectious diseases is tuberculosis. Tuberculosis or pulmonary TB is a direct infectious disease caused by TB bacteria, namely Mycobacterium tuberculosis. The majority of TB germs attack the lungs although it can also attack other organs of the body [18].

In healthy people, people infected with Mycobacterium tuberculosis often do not cause symptoms (no symptoms) because the person's immune system provides a defense against the bacteria. Active symptoms of pulmonary TB are cough, sometimes accompanied by sputum or blood, chest pain, weakness, weight loss, and fever and night sweats [9].

The source of transmission of the disease is from positive tuberculosis patients. When the positive patient is coughing or sneezing, the germs are spread into the air in the form of droplets containing Mycobacterium tuberculosis bacteria. These bacteria can survive in air at room temperature for several hours. A person can become infected if the droplets are inhaled into the respiratory tract. As long as the bacteria enters the human body through breathing, it can spread from the lungs to other body parts, through the circulatory system, lymphatic system, respiratory tract or direct spread to other body parts [18].

The transmission power of a patient is determined by the number of bacteria that are removed from the lungs. The higher the positive degree of sputum examination results, the more infectious the patient is.

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If the results of the sputum examination are negative, it is considered non-infectious. The likelihood of a person being infected with TB is determined by the concentration of droplets in the air and the duration of breathing the air [18].

Susceptibility to Mycobacterium tuberculosis is a factor that is determined by the risk of getting infection and the risk of developing clinical disease after infection occurs. Factors that affect the possibility of someone suffering from TB are low immune systems, including poor nutrition or HIV/AIDS, infant age, or the elderly who have a history of comorbidities [8].

According to WHO (2018), tuberculosis remains the 10th leading cause of death in the world and the prevalence of new cases is estimated at around 10 million people worldwide; as many as 5.7 million in men; 3.2 million in women; and 1.1 million in children. This global situation leads to a bad situation, thus WHO declares cases of tuberculosis as a global health emergency [19].

Tuberculosis cases in Indonesia reached 1,017,290 cases and in Gorontalo Province as many as 4,547 cases so that this province was ranked 32nd out of 34 provinces in Indonesia after West Papua Province (3,588 cases) and North Kalimantan (as many as 350,000 cases) [22].

Based on data from the Bone Bolango Health Office (2019), the Kabila Health Center is in the first place for tuberculosis cases, with a total of 86 patients. Based on data from the Kabila Health Center (2021), there were 13 cases of tuberculosis undergoing advanced treatment from April to May [5].

Tuberculosis can be cured by the use of Anti Tuberculosis Drugs (OAT) consisting of INH, rifampin, pyrazinamide, and ethambutol with Directly Observed Treatment, Short-course (DOTS) method. OAT treatment will leave residual lesions such as cavities, fibrosis, destroyed lung and so on which are predisposing factors to lung fungal infections [16]. Giving Anti Tuberculosis Drugs in the long term can suppress the growth of the body's normal flora so that the growth of opportunistic fungi is not inhibited [1].

The use of antibiotics in the long term is a factor that causes these fungi to grow. Because the use of antibiotics causes a deficiency of vitamin B12 and vitamin K produced by good bacteria that are killed with antibiotics in the long term so that the immune system in patients with pulmonary tuberculosis decreases and fungi will easily grow to become pathogens [8] causing an increase in the risk of pulmonary mycoses in patients with pulmonary tuberculosis.

The general symptoms of pulmonary mycoses are the same as other microbial infections including coughing or coughing up blood, a lot of phlegm, shortness of breath, fever, chest pain, but can also be asymptomatic. Pulmonary mycosis often accompanies other diseases and there are no typical symptoms hence often not diagnosed and the presence of fungi in the lungs is not known. Therefore laboratory tests are very important to identify the presence of fungi in sputum samples to conclude the cause of infection in the lungs [5]. One of the opportunistic fungi that often infects the lungs is Aspergillus sp. [11].

Aspergillus sp. is a type of fungus that belongs to the category of eukaryotic microorganisms. The microscopic characteristics of Aspergillus sp. are septate and branched hyphae, conidiophores emerge from foot cells (swollen and thick-walled mycelium) carrying stigmata. The conidia will grow in the form of green, brown or black colored chains. The macroscopic characteristics are fertile hyphae that appear on the surface and vegetative

hyphae that are below the surface. This fungus grows to form fibrous, smooth, convex mold colonies, and is uniform in gray, green, brown, black, or white in color. This color is influenced by the color of the spores, when the spores are green, the colony will be green and hence the color that was originally white becomes no longer visible [14].

Species of Aspergillus sp. are known to be ubiquitous, grow almost on all substrates, and belong to pathogenic fungi. Aspergillus sp. can produce mycotoxin called aflatoxin. Mycotoxins are identified as substances produced by fungi in foodstuffs, wherein fungi are resistant to heat. Aspergillus fungi, especially Aspergillus flavus and Aspergillus fumigatus can cause Aspergillosis, an inflammation of the granulomatosis of the bronchi, ears, or mucous membranes of the eyes, sometimes on the skin and subcutaneously on the bones, lungs and meninges [15].

Aspergillus sp. is a fungus that produces aflatoxin, a toxin that can kill humans because it can cause liver cancer if it enters the body through food. Various forms such as clinical and pathological changes of mycotoxicosis can be characterized by symptoms such as nausea, vomiting, abdominal pain, swollen lungs, seizures and even coma, and can cause death even though such cases are rare [15].

Aspergillus sp. lives in nature as a saprophyte and almost all media can grow this fungus, especially in the tropics with high humidity and the presence of predisposing factors that make it easier for the fungus to cause diseases. The entry of Aspergillus sp. fungal spores in humans are generally through inhalation where the incubation period cannot be known with certainty. Aspergillosis can affect anyone and all ages. A report states that the hospital environment is often contaminated with the spores. An increase in the number of Aspergillus sp spores can be found in the hospital construction and in the ventilation system. Even the area around the intravenous catheter can also be the entry point for Aspergillus sp [3].

Diagnosing an infection caused by Aspergillus depends on the type of fungal infection. Aspergillus is sometimes found in the saliva and phlegm of healthy people, so it is difficult to distinguish Aspergillus from other fungi under a microscope [8].

Fungal culture examination usually uses Potato Dextrose Agar (PDA) media. PDA is a medium that is rich in nutrients and is used for the growth of various fungi. Most fungi thrive on PDA, but sometimes fungi growing in this medium produce excess mycelia because the nutrient content of the medium is too rich for some fungi. PDA can be used by Ascomycota as a growth medium. PDA media contains 4.0 g/L PDA, 20.0 g/L Glucose, 15.09 g/L Agar, and 1 L Aquades [12].

Based on its composition, PDA media is classified as semi-synthetic media because it is composed of natural ingredients (potatoes) and synthetic materials (dextrose and agar). Potatoes are a source of carbon (carbohydrates), vitamins, and energy. Dextrose is a source of sugar and energy. Agar is a component that functions to compact the PDA medium. Each component is indispensable for the growth and proliferation of microorganisms, especially fungi [12]

Aspergillus has a habitat in the soil and is mostly found in dust and decaying organic matter. Aspergillus spores that are in the air are called conidia. Aspergillus conidia are small enough (2–3 m) in diameter to reach the alveoli. After these conidia are inhaled, they develop and the hyphae colonize in the bronchial branches. This causes the patient's sputum to contain the fungus Aspergillus [7].

Aspergillus sp. will be able to form colonization in the bronchi and lung cavities that arise due to pulmonary TB disease. The balls can be found in cavities called Aspergilloma. Aspergilloma is usually found in the upper logus of the lung with a diameter of several centimeters. Aspergilloma enters the lungs through the respiratory tract. Initially, it will appear a hole in the lung [6] then a ball of fungi will begin to grow in the hole. This disease often occurs in people who have lung problems such as tuberculosis or have a weakened immune system [7].

The purpose of this study was to determine the types of Aspergillus sp in pulmonary tuberculosis patients undergoing advanced treatment at the Kabila Health Center. Bone Bolango.

RESEARCH METHODS

The approach and type of research used in this research was a qualitative approach and a descriptive type of research with the aim of describing the morphological characteristics of the Aspergillus sp based on macroscopic and microscopic observations.

The type of data used were primary data and secondary data. In this study, the primary data was the result of examination of the Aspergillus sp at the Microbiology Laboratory, Bina Mandiri University, Gorontalo, while the secondary data was the result of observation of the medical records of pulmonary tuberculosis patients who were currently undergoing advanced treatment at the Kabila Health Center. Bone Bolango.

Sources of data in this study were obtained from answers from respondents through questionnaires and from the results of documentation.

The population in this study were all pulmonary tuberculosis patients who were currently undergoing advanced treatment at the Kabila Health Center. Bone Bolango, Gorontalo Province, in 2021 as many as 13 patients.

The sample in this study were pulmonary tuberculosis patients who were undergoing advanced treatment at the Kabila Health Center. Bone Bolango, as many as 13 patients.

The sampling technique in this study used the Total Sampling technique. Total sampling is a sampling technique where the number of samples is the same as the population. The reason for taking the total sampling was the population size is less than 30 patients.

The data in this study were analyzed descriptively, namely describing the morphological characteristics of the Aspergillus sp based on macroscopic and microscopic observations, then continued with identification activities, namely matching the characteristics of the fungi obtained from observations by referring to the Clinical Mycology and Pictorial Atlas of Soil and Seed Fungi book.

RESULTS

Based on the results of research conducted at the Microbiology laboratory of Bina Mandiri University, Gorontalo, from 13 sputum samples of pulmonary tuberculosis patients who were currently undergoing advanced treatment, four pure isolates were obtained.

1. Isolation 1

Fungal isolate 1 was obtained from patient code A1. The pure isolates obtained were identified and observed macroscopically and microscopically. From macroscopic observations of mold 4 isolates obtained the characteristics as shown in Table 1.

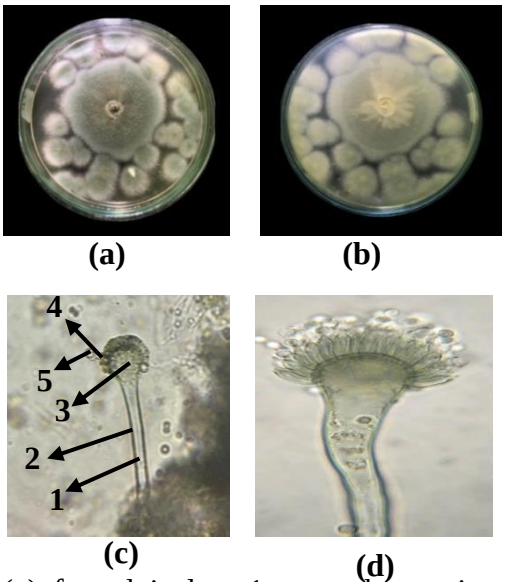
Tabel 1. Results of Macroscopic Observations of Isolates 1

No.	Characteristics	Results
1.	Color of colony	Green
2.	Base color on medium	Green
3.	Inverse color	Yellowish green

4.	Colony properties	Coarse powder

Source: Primary Data, 2021

For more details, macroscopic and microscopic observations on isolation media can be seen in Figure 1 as follows.



(a) **(b)** **(c)** **(d)**

(a) fungal isolate 1 seen above view; (b) fungal isolate 1 bottom view; (c) The results of microscopic observations of fungal isolates 1 at a magnification of 400 times; (d) comparison [5].

Based on microscopic observations made on fungal isolates 1 obtained the characteristics as shown in Table 2.

Tabel 2. Results of Microscopic Observations of Isolate 1

No.	Characteristics	Results
1.	Hyphae :	
	a. Septa/non	Non-insulated (asepta)
2.	Conidiophores:	
	a. Color	Hyaline
	b. Branched/non	Non
	c. Smooth wall/ coarse	Smooth
3.	Vesicle:	
	a. Shape	Round
	b. Existed / non	Existed
	c. color	Brown
4.	Fialida :	
	a. Growing place	Metula
	b. Color	Brown
	c. Shape	Round
5.	Conidia :	
	a. Color	Transparent
	b. Shape	Round
	c. Wall	Coarse

Source : Primary Data, 2021

From the characteristics of the macroscopic and microscopic examination, the fungal isolate of code 1 can be classified as follows:

Kingdom : Fungi
Division : Amastigomycota
Class : Eurotiomycetes
Ordo : Eurotiales
Family : Trichocomaceae
Genus : Aspergillus
Spesies : Aspergillus fumigatus [20].

2. Isolation 2

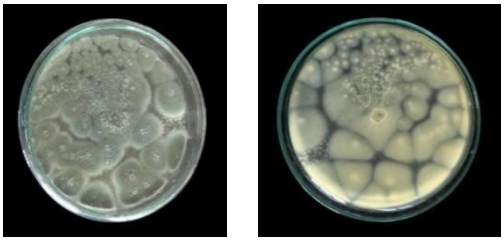
Fungal isolate 2 was obtained from patient code A2. The pure isolates obtained were identified and observed macroscopically and microscopically. From macroscopic observations obtained characteristics as in Table 3.

Tabel 3. Results of Macroscopic Observations of Isolate 2

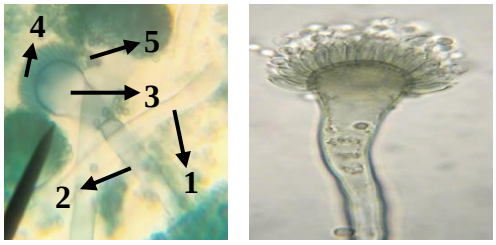
No.	Characteristics	Results
1.	Color of colony	Green
2.	Base color on medium	Green
3.	Inverse color	Yellowish green
4.	Colony properties	Coarse powder

Source: Primary Data, 2021

For more details, macroscopic and microscopic observations on isolation media can be seen in Figure 2 as follows:



(a) **(b)**



(c) **(d)**

Gambar 1. fungal isolate 2 seen above view; (b) fungal isolate 2 bottom view; (c) The results of microscopic observations of fungal isolates 2 at a magnification of 400 times; (d) comparison [5].

Based on microscopic observations made on fungal isolates 2 obtained the characteristics as shown in Table 4

Tabel 4. Results of Microscopic Observations of Isolate 2

No.	Characteristics	Results
1.	Hyphae :	
	a. Septa/non	Non-insulated (asepta)
2.	Conidiophores:	
	a. Color	Hyaline
	b. Branched/non	Non
	c. Smooth wall/ coarse	Smooth
3.	Vesicle:	
	a. Shape	Round
	b. Existed / non	Existed
	c. color	Brown
4.	Fialida :	
	a. Growing place	Metula Brown
	b. Color	Round
	c. Shape	
5.	Conidia :	
	a. Color	Transparent
	b. Shape	Round
	c. Wall	Coarse

Source: Primary Data, 2021

From the characteristics of the macroscopic and microscopic examination, the fungal isolate of code 2 can be classified as follows:

Kingdom : Fungi
Divisi : Amastigomycota
Kelas : Eurotiomycetes
Ordo : Eurotiales
Family : Trichocomaceae
Genus : Aspergillus
Spesies : Aspergillus fumigatus [20].

3. Isolation 3

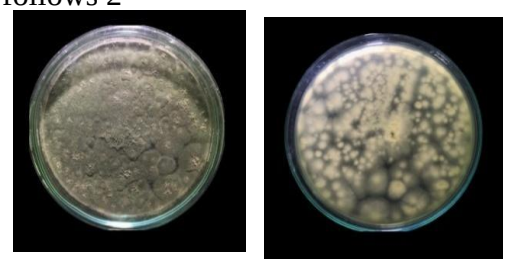
Fungal isolate 3 was obtained from patient code A3. The pure isolates obtained were identified and observed macroscopically and microscopically. From macroscopic observations obtained characteristics as in Table 5.

Tabel 5. Results of Macroscopic Observations of Isolate 3

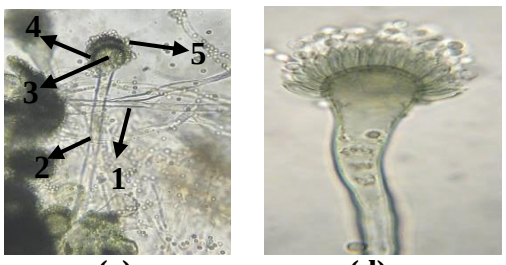
No.	Characteristics	Results
1.	Color of colony	Green
2.	Base color on medium	Green
3.	Inverse color	Yellowish green
4.	Colony properties	Coarse powder

Source: Primary Data, 2021

For more details, macroscopic and microscopic observations on isolation media can be seen in Figure 3 as follows 2



(a) **(b)**



(c) **(d)**

Gambar 2. fungal isolate3 seen above view; (b) fungal isolate 3 bottom view; (c) The results of microscopic observations of fungal isolates 3 at a magnification of 400 times; (d) comparison [5].

Based on microscopic observations made on fungal isolates 2 obtained the characteristics as shown in Table 6

Tabel 6. Results of Microscopic Observations of Isolate 3

No.	Characteristics	Results
1.	Hyphae :	
	a. Septa/non	Non-insulated (asepta)
2.	Conidiophores:	
	a. Color	Hyaline
	b. Branched/non	Non
	c. Smooth wall/ coarse	Smooth
3.	Vesicle:	
	a. Shape	Round
	b. Existed / non	Existed
	c. color	Brown
4.	Fialida :	
	a. Growing place	Metula
	b. Color	Brown
	c. Shape	Round
5.	Conidia :	
	a. Color	Transparent
	b. Shape	Round
	c. Wall	Coarse

Source: Primary Data, 2021

From the characteristics of the macroscopic and microscopic examination, the fungal isolate of code 3 can be classified as follows:

Kingdom : Fungi
Divisi : Amastigomycota
Kelas : Eurotiomycetes
Ordo : Eurotiales
Family : Trichocomaceae
Genus : Aspergillus
Spesies : Aspergillus fumigatus [20].

4. Isolation 4

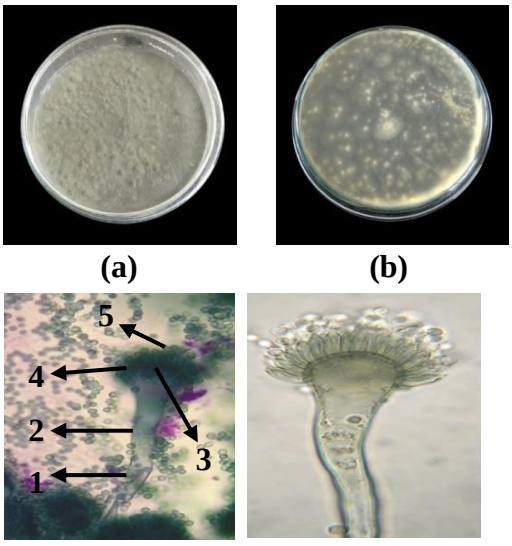
Fungal isolate 4 was obtained from patient code A4. The pure isolates obtained were identified and observed macroscopically and microscopically. From macroscopic observations obtained characteristics as in Table 7.

Tabel 7. Results of Macroscopic Observations of Isolate 4

No.	Characteristics	Results
1.	Color of colony	Green
2.	Base color on medium	Green
3.	Inverse color	Yellowish green
4.	Colony properties	Coarse powder

Source: Primary Data, 2021

For more details, macroscopic and microscopic observations on isolation media can be seen in Figure 4 as follows:



(a) **(b)** **(c)** **(d)**

Gambar 3. fungal isolate 4 seen above view; (b) fungal isolate 4 bottom view; (c) The results of microscopic observations of fungal isolates 4 at a magnification of 400 X; (d) comparison [5].

Based on microscopic observations made on fungal isolates 4 obtained the characteristics as shown in Table 8

Tabel 8. Results of Microscopic Observations of Isolate 4

No.	Characteristics	Results
1.	Hyphae :	
	a. Septa/non	Non-insulated (asepta)
2.	Conidiophores:	
	a. Color	Hyaline
	b. Branched/non	Non
	c. Smooth Wall/ coarse	Smooth
3.	Vesicle:	
	a. Shape	Round
	b. Existed / non	Existed
	c. Color	Brown
4.	Fialida :	
	a. Growing place	Metula
	b. Color	Brown
	c. Shape	Round
5.	Conidia :	
	a. Color	Transparent
	b. Shape	Round
	c. Wall	Coarse

Source: Primary Data, 2021

From the characteristics of the macroscopic and microscopic examination, the fungal isolate of code 7 can be classified as follows:

Kingdom : Fungi
Divisi : Amastigomycota
Kelas : Eurotiomycetes
Ordo : Eurotiales
Family : Trichocomaceae
Genus : Aspergillus
Spesies : Aspergillus fumigatus [20].

DISCUSSION

Based on the results of research conducted at the Microbiology laboratory of Bina Mandiri University, Gorontalo, from 13 sputum samples belonging to pulmonary tuberculosis patients who were currently undergoing advanced treatment, four pure isolates were obtained, including the Aspergillus fumigatus.

Aspergillus sp. is a fungus that belongs to the Ascomycetes class. It is widely distributed in nature; it can be found in soil, on decaying vegetation, and in various organic materials. Accidental inhalation of dust contaminated with spores is the most common way humans are infected with the fungus which can lead to sinusitis and bronchopulmonary disease [18].

The filaments of Aspergillus are long branched and form mycelia and conidiospores in the culture medium. Aspergillus reproduces through the formation of hyphae or buds and produces spore-forming conidiophores. The spores are freely dispersed in the open air so that inhalation into the body through the respiratory tract is unavoidable [15].

Pathogenic fungi are microorganisms that can cause disease in humans because fungi grow cosmopolitan with humans both on soil and in water. Diseases by fungi occur due to fungal infections in the human body. Fungal infections are caused by colonization of fungal spores in organs or in certain parts of the body Infections caused by fungi are called mycosis. Mycosis of the respiratory system can occur in the upper and lower respiratory tract. The most common mycosis found in the lower respiratory tract is pulmonary tuberculosis [17].

Lung fungal infections were more often caused by opportunistic fungal infections Aspergillus. Some of these fungal opportunistic infections are ubiquitous and often infect patients with extensive or long-term use of antibiotic drugs in addition to the emergence of other predisposing factors [7].

Most patients with opportunistic infections suffer from serious illnesses and have compromised immune systems. Opportunistic infections can also be suffered by immunocompetent

individuals. During infection, most patients produce significant humoral and cellular immune responses to fungal antigens [19].

In patients with pulmonary tuberculosis with anatomical defects of the lung accompanied by the administration of anti-tuberculosis drugs for a long time will suppress the normal flora so that the growth of opportunistic fungi is not inhibited. Chronic pulmonary tuberculosis also predisposes to invasive pulmonary aspergillosis. If a person inhales large amounts of Aspergillus fungal spores, it can cause acute pneumonitis and lung cancer [2].

Aspergillus fumigatus is one of the saprophytic fungi, especially related to soil and vegetable material decay. Aspergillus fumigatus in nature grows as a mass of branched hyphae, but also produces a large number of asexual spores, known as conidia (in this structure known as conidiaphores). Conidia are released into the environment and can be carried with the air so that they become a means to contaminate food and water sources. Conidia concentrations in the air can range from 1 to 100 per meter^3. Therefore, it can be routinely inhaled by humans and has a diameter small enough (2–3 m) to reach the alveoli. After these conidia are inhaled, they develop and the hyphae colonize in the bronchial branches. This causes the sputum in tuberculosis patients to contain Aspergillus fumigatus [6].

Aspergillus fumigatus will be able to colonize the bronchi and lung cavities that arise due to pulmonary TB disease [7]. Aspergillus fungus balls can be present in the cyst cavity or cavity. This ball of Aspergillus is called Aspergilloma. Aspergilloma is usually found in the upper logus of the lung with a diameter of several centimeters. Aspergilloma enters the lungs through the respiratory tract. In the early stages, holes will appear in the lungs. Then a mushroom ball will start growing in the hole. This disease often occurs in people who have lung problems such as tuberculosis or have a weakened immune system [7].

Well-treated tuberculosis cavities sometimes remain open and can become infected with Aspergillus fumigatus [5]. On the X-ray, you can see a ball consisting of a fungus in the cavity. This condition sometimes causes severe hemoptysis and can even be fatal. Pulmonary aspergillosis is usually a secondary disease in patients with chronic disorders such as tuberculosis. This disease is caused by the Aspergillus sp, especially Aspergillus fumigatus species.

This fungus is widely scattered in the air so that it is easily inhaled through the respiratory tract. Inhaled fungal spores then colonize the mucosal surface. Fungi can penetrate the tissue only when there is a disturbance of the immune system, either local or systemic [19].

This fungus will infect patients with chronic disorders such as tuberculosis. Another factor that affects the growth of this fungus is the patient's daily activities. Based on the patient's observations and confessions, patients who were positively infected with Aspergillus sp. were smokers. Smoking habits can increase susceptibility to infection. Aspergillus sp. fungus found in the air will be easily inhaled and cause infection due to smoking habits. Lung abnormalities in the form of cavities in the lungs caused by tuberculosis and smoking habits increase the risk of being easily exposed to this fungus [11].

Several diseases increase the risk of pulmonary mycoses and play an important role in the diagnostic criteria for pulmonary mycoses. In the Diagnostic Guidelines, most pulmonary mycoses are asymptomatic on chest X-ray, interstitial infiltrates, consolidation, multiple nodules, cavities, and pleural effusions

can be found. The typical chest radiograph is a fungus ball in the cavity of an aspergilloma. Better results were obtained on a chest CT scan. A routine laboratory result that may be associated with pulmonary mycoses is an elevated eosinophil cell count [19].

Mycological examination is a very important diagnostic procedure for pulmonary mycoses. The quality of this examination is determined by the selection, collection and delivery of the specimen. Inadequate handling of specimens can result in inaccurate diagnosis. Specimens can be obtained from sputum, bronchial lavage, broncho-alveolar lavage (BAL), biopsy tissue, blood, pus and others [7].

Prevention of pulmonary mycoses can use masks and protective clothing to reduce contact with spores. Avoid travel to/in endemic areas. Immunization usually does not provide effective results. Patients undergoing bone marrow transplantation or prolonged periods of neutropenia are advised to avoid activities (e.g., gardening, cleaning, agitating debris) or objects (e.g., potted plants, flowers, fresh fruit, vegetables) that may cause exposure to Aspergillus spores. or other mushrooms. For patients undergoing bone marrow transplantation, solid organ transplantation, or antileukemic chemotherapy, use an air filtration system in the treatment unit to minimize the patient's risk of exposure to Aspergillus spores. The use of prophylactic antifungal therapy is recommended in patients who are at high risk for opportunistic fungal infections, including patients with a history of fungal infections [7].

CONCLUSION

Based on the results of research conducted at the Microbiology laboratory of Bina Mandiri University, Gorontalo, from 13 sputum samples belonging to pulmonary tuberculosis patients who were currently undergoing advanced treatment at the Kabila Health Center, Bone Bolango Regency, four pure isolates were found which belonged to the fungus Aspergillusfumigatus..

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