INDOGENIUS

ISSN 2828-1365

Volume 4, Issue 3 : 753 - 759

Overview of Fungi in the Sputum of Pneumonia Patients at Bendan Regional General Hospital, Pekalongan City

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Article Info

Keywords :
Pneumonia, Sputum, Fungi, Gram, KOH, LPCB

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ABSTRACT

Background & Objective: Pneumonia remains one of the leading causes of morbidity and mortality, particularly among vulnerable groups such as children, the elderly, and immunosuppressed patients. In addition to being caused by bacteria and viruses, fungal infections can also exacerbate a patient's condition, but they are often overlooked because they are not always the primary focus of laboratory testing. This study aims to describe the morphology of fungi in the sputum of pneumonia patients at Bendan General Hospital in Pekalongan City as initial data that can be used to support earlier diagnosis of fungal infections. **Method:** This study is descriptive in nature, using direct microscopic examination with three methods: Gram staining, Lactophenol Cotton Blue (LPCB), and 10% Potassium Hydroxide (KOH). Sputum samples were collected purposively from pneumonia patients and examined at the AAK Pekalongan Microbiology Laboratory. **Result:** Out of 26 samples, 17 were positive for fungi (65.4%) using the Gram and LPCB methods. The KOH method showed 5 positive samples (19.2%). The fungal morphology observed included yeast cells (blastospores) and branched hyphae, consistent with the characteristics of fungi from the genus *Candida spp*. **Conclusion:** Microscopic examination using Gram and LPCB methods provided a more easily observable descriptive morphology of fungi, such as yeast cells and branched hyphae. Meanwhile, the KOH method yielded lower results (19.2%), possibly due to limitations in preparation techniques or the nature of the reagents, which did not clearly display fungal structures as effectively as other methods using additional stains. These findings emphasize the importance of direct microscopic examination as an initial step that can help identify the presence of opportunistic fungi in

pneumonia patients and support clinical diagnosis considerations in the laboratory.

DOI: https://doi.org/10.56359/igj.v4i3.654

Introduction

Pneumonia is an infectious disease of the lower respiratory tract that affects lung tissue and can be caused by bacteria, viruses, or fungi (Ministry of Health, 2020). Direct fungal examination is an important part of medical mycology for laboratory diagnosis (Indrayati et al., 2018). In Indonesia, pneumonia remains one of the leading causes of death, particularly among children and the elderly (WHO, 2021). One of the challenges in managing pneumonia is when the infection is caused by fungi, especially in patients with compromised immune systems such as those with HIV/AIDS, cancer patients, or those undergoing immunosuppressive therapy.

Opportunistic fungal infections in the respiratory tract are often undetected due to the limited additional tests conducted in healthcare facilities. However, the presence of fungi such as *Candida spp*. and *Aspergillus spp*. can worsen the clinical condition of patients. Previous studies have highlighted the importance of microscopic fungal examination in sputum. A study by Angriani et al. (2019) identified *Aspergillus fumigatus* in the sputum of pulmonary TB patients, while Akbar (2024) reported the dominance of *Candida albicans* in TB patients. These findings indicate that opportunistic fungi can be found in the respiratory tract, even in patients without primary fungal infections. Direct microscopic examination is also effective as an initial screening method for detecting fungal morphology in sputum (Yusuf et al., 2020).

Researchers were motivated to conduct this study due to the limited local data describing the presence of fungi in the sputum of pneumonia patients, particularly at RSUD Bendan in Pekalongan City. Additionally, Gram staining, KOH, and Lactophenol Cotton Blue (LPCB) are simple, rapid, and commonly used direct microscopic techniques in microbiological laboratory examinations. Direct microscopy is also an essential basic technique in clinical laboratories for the early detection of fungal infections (Sutrisno & Lestari, 2020). It is hoped that the results of this study can add scientific information regarding the role of fungal examination in supporting laboratory-based pneumonia diagnosis. Morphological structures such as blastospores and branched hyphae can be observed characteristically through microscopic examination (Nugroho, 2019).

Objective

This study aims to determine the morphological characteristics of fungi in the sputum of pneumonia patients at Bendan Regional General Hospital in Pekalongan City through direct microscopic examination using Gram staining, Lactophenol Cotton Blue (LPCB), and 10% KOH.

Method

This study is a descriptive cross-sectional study aimed at describing the morphology of fungi in the sputum of pneumonia patients. The main focus of the study is direct microscopic examination using three methods, namely Gram staining, Lactophenol Cotton Blue (LPCB), and 10% Potassium Hydroxide (KOH).

The study population consists of all pneumonia patients who underwent sputum examination at Bendan General Hospital in Pekalongan City from February to April 2025. Sampling was conducted using purposive sampling, with a total of 26 sputum samples meeting the inclusion criteria: productive sputum, not excessively mixed with blood, and from patients clinically diagnosed with pneumonia.

The examination was conducted at the Microbiology Laboratory of the Pekalongan Health Analyst Academy (AAK). Research instruments included microscope slides, coverslips, a light microscope, pipettes, loops, and Gram stain, LPCB, and 10% KOH staining materials. Each sample was examined microscopically according to the method used:

1. Gram staining to detect yeast cells and hyphae using differential staining,
2. LPCB to clarify morphological details such as blastospores and branched hyphae,
3. 10% KOH to dissolve mucus and facilitate observation of fungal structures,

The data obtained were analyzed descriptively and presented in tables showing the number and percentage of positive and negative samples from each method (Gram, LPCB, and KOH).

The study was conducted from February to April 2025 at the Bendan General Hospital in Pekalongan City as the sputum sample collection site, while the examinations were performed at the Microbiology Laboratory of the Health Analyst Academy (AAK) in Pekalongan.

Results

The results of direct microscopic examination of 26 sputum samples from pneumonia patients showed variations in the number of positive findings based on the staining method used. This examination included three main methods: Gram, Lactophenol Cotton Blue (LPCB), and 10% KOH. Each method produced different morphological patterns of fungi, and the results were documented in the following microscopic images:

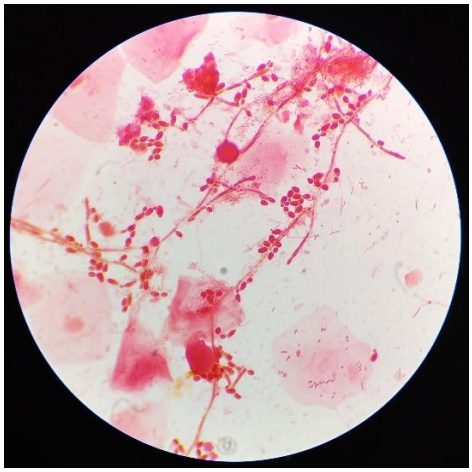

FIGURE 1. Gram Stain – Morphology of *Candida sp.* Fungi in the Form of Hyphae and Blastospores (Yeast)

Figure 1. shows the results of examination using Gram staining. The structure of blastospores and hyphae can be seen with a reddish-purple color, which is a characteristic of Gram differential staining. This stain allows for contrasting visualization of the fungal cell wall.

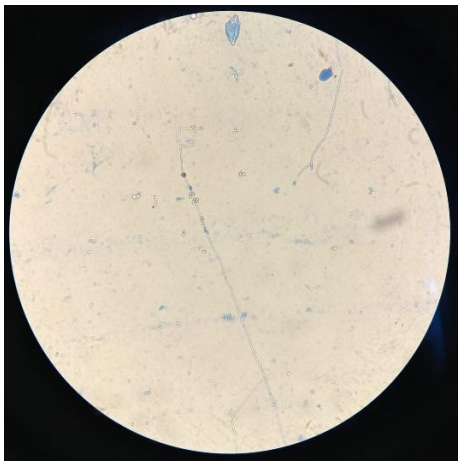

FIGURE 2. LPCB Examination – Morphology of *Candida sp*. Fungi in the Form of Hyphae and Blastospores (Yeast)

Figure 2. shows the results of the examination using the LPCB method. The bright blue color clearly highlights the morphology of the blastospores and branched hyphae, as LPCB works by killing the fungi and providing high contrast.

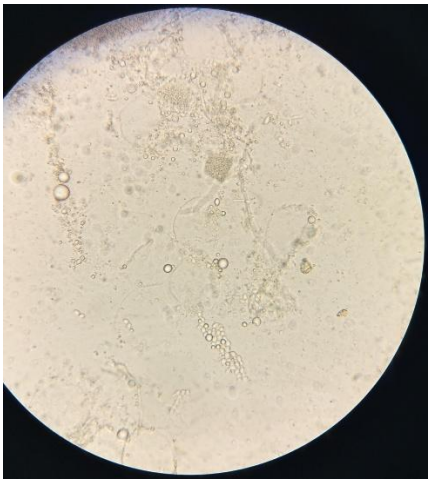

FIGURE 3. 10% KOH examination – Morphology of *Candida sp*. fungi in the form of hyphae and blastospores (yeast)

Figure 3. shows the results of the 10% KOH method. The fungal structure appears duller and less contrasting, but the mucus from the sputum has been successfully dissolved, so that some blastospore and hyphae structures can still be observed.

The examination procedure is carried out by placing the sputum sample on a glass slide, then:

1. For Gram staining, fixation is performed, followed by the addition of crystal violet, iodine, alcohol, and safranin. After drying, immersion oil is added, and the sample is observed under a light microscope at 1000x magnification.
2. For LPCB, the sputum is directly mixed with the staining solution on the slide, then observed under a microscope at 10x to 40x magnification.
3. For KOH, sputum is dripped with 10% KOH and left for several minutes to dissolve the mucus, then observed using a microscope at 10x to 40x magnification.

From a total of 26 sputum samples examined, the results are shown in the table below:

TABLE 1. Results of Fungal Examination of Patient Sputum Pneumonia at Bendan Regional General Hospital, Pekalongan City

Test Method	Positive Samples	Negative Samples	Percentage (%) Positive
Gram	17	9	65.4%
LPCB	17	9	65.4%
KOH	5	21	34,6%

TABLE 2. Summary of the Percentage of Mushroom Examination Results in the Sputum of Pneumonia Patients at Bendan Regional General Hospital, Pekalongan City

Sample Results	Total	Percentage (%)
Positive	17	65.4%
Negative	9	34,6%

Based on the two tables above, the Gram stain results showed that out of 26 samples, 17 (65.4%) were positive for yeast cells and/or hyphae identified as *Candida sp.* Nine samples (34.6%) did not show the presence of fungi. The LPCB method also yielded the same results, with 17 samples (65.4%) testing positive for *Candida sp*. and 9 samples (34.6%) testing negative. Meanwhile, the 10% KOH method showed positive results in 5 samples (19.2%) and negative results in 21 samples (80.8%).

Most of the positive samples showed fungal morphology in the form of yeast cells (blastospores) and branched hyphae. Gram staining revealed fungal cell walls with a reddish-purple color, while the LPCB method displayed blastospores and branched hyphae with sharp blue contrast. In the KOH examination, only faint fungal structures were visible due to the absence of staining, but the sputum mucus was successfully dissolved to facilitate observation. Based on the morphological characteristics observed, the findings were consistent with the typical appearance of fungi from the genus *Candida spp*.

Additionally, the quality of the results can be influenced by the thickness of the preparation, fixation technique, and the number of fungal elements in the sputum. Preparations that are too thick or suboptimal staining can cause fungal elements to be unclear. Thus, of the three methods used, Gram staining and LPCB provide a clearer microscopic view of fungal morphology compared to the KOH method.

Discussion

This study aims to describe the morphology of fungi in the sputum of pneumonia patients using three direct microscopic methods: Gram, Lactophenol Cotton Blue (LPCB), and 10% KOH. Each method is a variable in the study with different working principles and advantages, thereby providing diverse insights into the morphological structure of fungi, particularly those belonging to the genus *Candida spp*.

In the Gram and LPCB methods, 17 positive samples were found out of a total of 26 (65.4%), while the KOH method only showed 5 positive samples (19.2%). These findings indicate that Gram and LPCB staining are more effective in visually revealing fungal morphology such as yeast cells (blastospores) and branched hyphae. The clearly stained hyphae and blastospores in the Gram and LPCB methods make them superior to KOH in visually describing fungi.

The Gram method works based on differential staining that highlights cell wall structures, while LPCB provides high blue color contrast and immobilization effects

on fungi, making structures appear sharper. Conversely, the KOH method only dissolves mucus in sputum and does not provide staining, causing fungal structures to appear dimmer and less distinct.

This study is supported by Angriani et al. (2019), who identified *Aspergillus fumigatus* in sputum from pulmonary TB patients, and Akbar (2024), who reported the dominance of *Candida albicans* in TB patients. Similar findings were also reported by Maulida and Wulandari (2021), who noted that the LPCB method is highly effective in displaying blastospore structures in immunocompromised patients. Additionally, Pratiwi and Lestari (2020) emphasized the important role of LPCB as a high-contrast stain in fungal examinations.

From these results, it can be concluded that Gram staining and LPCB have better sensitivity in depicting fungal structures compared to KOH. This aligns with the research by Sari et al. (2022), who stated that the Gram method can serve as an effective initial screening tool for fungi in sputum. The findings of Widarti et al. (2023) also reinforce that respiratory tract infections caused by opportunistic fungi such as *Candida spp*. are commonly found, especially in patients with immune disorders.

In this context, direct microscopic examination serves as a reliable initial method, especially in healthcare facilities with limited access to advanced techniques such as culture or PCR. As stated by Yusuf et al. (2020), this method is suitable as an initial approach for detecting fungi in clinical laboratories.

Strengths of the study:

- Three direct microscopic methods commonly used in clinical laboratories were employed.
- Examination was performed directly on sputum samples without the need for culture, resulting in faster results.
- The three methods provide a comprehensive descriptive visual comparison.

Limitations of the study:

- No confirmation was performed using culture or PCR, so identification was based solely on morphology.
- Samples were limited to one hospital and a short collection period (February–April 2025).
- Some samples did not meet optimal criteria due to sputum viscosity or uneven staining.

Considering these strengths and limitations, this study still demonstrates that the Gram and LPCB methods can serve as practical alternatives for initial fungal testing in pneumonia patients, while also opening opportunities for more complex follow-up research.

Conclusion

Microscopic examination using Gram and LPCB staining provides a more easily observable descriptive morphological picture of fungi, such as yeast cells and branched hyphae. Meanwhile, the KOH method yielded lower results (19.2%), possibly due to limitations in the preparation technique or the nature of the reagent, which does not display fungal structures as clearly as other methods using additional stains. These findings emphasize the importance of direct microscopic examination as an initial step that can help identify the presence of opportunistic fungi in pneumonia patients and support clinical diagnosis considerations in the laboratory.

Further research is recommended using confirmatory methods such as culture or polymerase chain reaction (PCR) to clarify the fungal species found and provide more specific information in the diagnosis of respiratory fungal infections.

Acknowledgement

The authors would like to thank the Bendan General Hospital in Pekalongan City and the AAK Pekalongan Microbiology Laboratory for the permission and facilities provided during the conduct of this study.

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