



IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS (MTB) IN SPUTUM OF PATIENTS WITH COUGH \geq 2 WEEKS USING ZIEHL-NEELEN STAINING METHOD AT RSUD SITI FATIMAH AZ-ZAHRA PALEMBANG IN 2025

IDENTIFIKASI MYCOBACTERIUM TUBERCULOSIS (MTB) DALAM SPUTUM PASIEN DENGAN BATUK \geq 2 MINGGU MENGGUNAKAN METODE PENCELUPAN ZIEHL-NEELEN DI RSUD SITI FATIMAH AZ-ZAHRA PALEMBANG PADA TAHUN 2025

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DOI: <https://doi.org/10.62567/micjo.v3i1.2330>

Abstract

Tuberculosis remains a major public health problem in Indonesia with high morbidity and mortality rates. This study aimed to identify Mycobacterium Tuberculosis (MTB) in sputum samples of patients with productive cough lasting \geq 2 weeks using the Ziehl-Neelsen staining method at RSUD Siti Fatimah Az- Zahra Palembang. This study uses a descriptive observational method with a cross-sectional approach and involves 30 respondents. The results showed that all respondents experienced cough, with the majority 24 respondents (80.0%) having productive cough \geq 2 weeks and 6 respondents (20.0%) having prolonged cough $<$ 2 weeks. Sputum examination revealed 17 respondents (56.7%) were Acid-Fast Bacilli (AFB) positive, while 13 respondents (43.3%) were negative. These findings indicate that a prolonged cough lasting \geq 2 weeks is associated with AFB-positive results, although tuberculosis may also occur in patients with prolonged cough lasting $<$ 2 weeks. It is recommended that patients with positive results undergo complete DOTS therapy, hospitals improve diagnostic services and health education, communities adopt a healthy lifestyle, and further research be conducted to explore the underlying causes of prolonged cough.

Keywords : Tuberculosis, Cough \geq 2 weeks, Sputum, Ziehl-Neelsen, AFB.

Abstrak

Tuberkulosis masih menjadi masalah kesehatan utama di Indonesia dengan angka kesakitan dan kematian yang tinggi. Penelitian ini bertujuan untuk mengidentifikasi Mycobacterium Tuberculosis (MTB) pada sputum penderita batuk berdahak \geq 2 minggu melalui metode pewarnaan Ziehl-Neelsen di



RSUD Siti Fatimah Az-Zahra Palembang. Penelitian ini menggunakan metode deskriptif observasional dengan pendekatan cross-sectional dan melibatkan 30 responden. Hasil penelitian menunjukkan bahwa seluruh responden mengalami batuk, dengan mayoritas 24 responden (80,0%) batuk berdahak ≥ 2 minggu dan 6 responden (20,0%) batuk berdahak < 2 minggu. Dari pemeriksaan sputum, 17 responden (56,7%) positif Basil Tahan Asam (BTA) dan 13 responden (43,3%) negatif. Temuan ini menunjukkan bahwa batuk berdahak ≥ 2 minggu memiliki hubungan dengan hasil pemeriksaan BTA positif, namun tidak menutup kemungkinan adanya kasus tuberkulosis pada pasien dengan batuk berdahak < 2 minggu. Disarankan agar pasien positif segera menjalani terapi DOTS secara tuntas, pihak rumah sakit meningkatkan pelayanan serta edukasi, masyarakat menerapkan pola hidup sehat, dan penelitian selanjutnya dilakukan untuk menggali lebih dalam faktor penyebab batuk berkepanjangan.

Kata Kunci : Tuberkulosis, Batuk ≥ 2 minggu, Sputum, Ziehl-Neelsen, BTA.

1. INTRODUCTION

Pulmonary disease known as Tuberculosis (TB) is caused by the bacterium *Mycobacterium tuberculosis* (MTB). This disease is typically transmitted through the air and attacks the respiratory tract in the lungs. When an infected person coughs or sneezes, the bacteria are dispersed into the air in the form of droplet nuclei, which commonly occurs in TB patients with positive Acid-Fast Bacilli (AFB) test results (H. Lestari, 2024).

Several common symptoms of pulmonary TB include unexplained weight loss for three consecutive months, fever accompanied by chills for more than one month, persistent cough lasting more than two weeks, chest pain, shortness of breath, decreased appetite, prolonged fatigue (malaise), night sweats despite the absence of physical activity, and blood-streaked sputum (M. Sabir & Sarifuddin, 2023). According to WHO data (2025), Tuberculosis (TB) is a global disease. In 2023, the WHO Southeast Asia Region recorded the highest number of new TB cases, accounting for 45%, followed by the African Region at 24% and the Western Pacific Region at 17%. Approximately 87% of the total global TB cases, with more than two-thirds of cases, were concentrated in Bangladesh, China, the Democratic Republic of the Congo, India, Nigeria, and the Philippines. Indonesia ranks second among countries with the highest TB burden worldwide (Lailatul & Ziyadatur, 2024).

After India, Indonesia has the second-highest number of TB cases, totaling 969,000 cases with 150,000 deaths. However, only 45.7% of these cases have been detected, while the remaining 54.3% remain undetected and unreported (Alif R. Bagaskara et al., 2024). According to the 2022 South Sumatra Provincial Health Profile, based on the Case Notification Rate (CNR), the number of pulmonary TB patients with positive AFB results reached 18,122 cases, an increase compared to 13,514 cases in 2021. This figure reflects the number of TB cases treated and reported per 100,000 population, indicating trends in TB incidence over time. The new case detection rate reached 53.7%, increasing from 40.1% in 2021; however, this figure remains far below the national target of 90% (Jannah et al., 2024).

Detection of *Mycobacterium tuberculosis* (MTB) in sputum can be performed using several methods, including Polymerase Chain Reaction (PCR), microscopic examination using Ziehl-Neelsen staining, and bacterial culture. PCR techniques have very high sensitivity in



detecting TB bacteria. Meanwhile, microscopic examination using the Ziehl–Neelsen staining method is the simplest, most efficient, and specific technique that can be applied in various laboratory facilities (Ramadhan et al., 2017).

Early diagnosis and monitoring of pulmonary TB treatment rely heavily on microscopic examination of Acid-Fast Bacilli (AFB) in sputum. To obtain accurate results, proper procedures are required, starting from sputum collection methods to the selection of appropriate samples and preparation of smears and sera for microscopic examination. The Ziehl–Neelsen staining method is one of the most commonly used techniques to identify TB, especially in areas with limited facilities and equipment for bacterial culture. This staining method enables the detection of

AFB using a microscope (Utami et al., 2021). It is a rapid method and offers the possibility of detecting cases with high bacterial loads and high transmission risk.

Sputum staining is an important technique in diagnosing pulmonary TB. Although analysis of sputum stained with Acid-Fast Fuchsin (AFF) is rapid and simple, it may lead to problems and inaccurate results in certain clinical situations. On the other hand, Ziehl–Neelsen staining is more accessible but has limitations, as many factors can affect the sensitivity and specificity of the results. The sensitivity of Ziehl–Neelsen staining in sputum smear preparations is influenced by the bacterial load and has been evaluated in various studies, showing a wide variation ranging from 22% to 80%. However, in cases of extrapulmonary TB, HIV co-infection, or pediatric TB, the bacterial load may be lower than required for optimal Ziehl–Neelsen staining results (Zaporojan et al., 2024).

Based on the background above, this study aims to conduct research entitled “Identification of Mycobacterium tuberculosis (MTB) in Sputum of Patients with Cough \geq 2 Weeks Using Ziehl–Neelsen Staining at RSUD Siti Fatimah Az-Zahra Palembang, 2025.”

2. RESEARCH METHOD

This study is a descriptive observational study with a cross-sectional approach (Amelia et al., 2023), aimed at observing the relationship between patients with a cough lasting \geq 2 weeks as the independent variable and sputum conversion as the dependent variable. All data were collected at a single point in time without any intervention, providing a snapshot of the current condition. The research was conducted quantitatively in a natural setting, using questionnaires and laboratory examinations to detect the presence of Mycobacterium tuberculosis (MTB) in patients’ sputum samples through the Ziehl–Neelsen staining method. The study was carried out at RSUD Siti Fatimah Az-Zahra Palembang in 2025, with respondents experiencing productive cough for \geq 2 weeks as the study subjects. The results of this study are expected to provide preliminary information to support early detection and management of tuberculosis cases in healthcare facilities.



3. RESULT AND DISCUSSION

a. Research Results

1) Univariate Analysis

This analysis aims to determine the frequency distribution and percentage of the dependent variable (results of MTB identification: positive/negative) and the independent variables (productive cough ≥ 2 weeks and sputum examination using Ziehl–Neelsen staining).

✓ Productive Cough ≥ 2 Weeks

In this study, the variable of productive cough ≥ 2 weeks was classified into two categories: productive cough ≥ 2 weeks and productive cough < 2 weeks. The detailed results are presented in Table 5.1 below:

Table 1. Frequency Distribution and Percentage of Respondents Based on Productive Cough ≥ 2 Weeks at RSUD Siti Fatimah Az-Zahra Palembang, 2025

| No | Cough with Phlegm for ≥ 2 Weeks | Frequency (N) | Percentage (%) |
|-------|--------------------------------------|---------------|----------------|
| 1 | Yes | 24 | 80.0 |
| 2 | No | 6 | 20.0 |
| Total | | 30 | 100 |

Based on Table 1 above, the frequency distribution and percentage of respondents show that out of a total of 30 patient samples, 24 respondents (80.0%) experienced productive cough for ≥ 2 weeks, while 6 respondents (20.0%) experienced productive cough for < 2 weeks.

b. Results of Ziehl–Neelsen Staining

In this study, the results of Ziehl–Neelsen staining were grouped into two categories: positive (Acid-Fast Bacilli/AFB detected in sputum specimens) and negative (no AFB detected in sputum specimens). For more detailed results, see Table 5.2 below:

Table 2. Frequency Distribution and Percentage of Respondents Based on Ziehl–Neelsen Staining Results at RSUD Siti Fatimah Az-Zahra Palembang, 2025

| No | Ziehl–Neelsen Staining Results | Frequency (N) | Percentage (%) |
|-------|--------------------------------|---------------|----------------|
| 1 | Positive | 17 | 56.7 |
| 2 | Negative | 13 | 43.3 |
| Total | | 30 | 100 |



Based on Table 5.2 above, the frequency distribution and percentage of respondents show that out of a total of 30 patient samples, 17 respondents (56.7%) had positive Acid-Fast Bacilli (AFB) staining results for tuberculosis, while 13 respondents (43.3%) had negative results.

c. Bivariate Analysis

This analysis was conducted to assess the relationship between the results of Acid-Fast Bacilli (AFB) examination using the Ziehl–Neelsen staining method and the duration of productive cough ≥ 2 weeks among respondents. The objective was to determine whether the duration of productive cough ≥ 2 weeks is significantly associated with positive pulmonary tuberculosis results based on AFB examination at RSUD Siti Fatimah Az-Zahra Palembang in 2025.

The analysis in this study employed the chi-square statistical test with a significance level set at $\alpha = 0.05$. If the $p\text{-value} \leq 0.05$, it can be concluded that there is a statistically significant relationship between the dependent variable and the independent variable.

✓ Identification of Ziehl–Neelsen Staining Results with the Incidence of Productive Cough ≥ 2 Weeks

The relationship between the results of Ziehl–Neelsen staining and the incidence of productive cough ≥ 2 weeks is presented in Table 5.3 as follows:

Table 3. Frequency and Percentage of Respondents Based on Ziehl–Neelsen Staining Results and the Incidence of Productive Cough ≥ 2 Weeks at RSUD Siti Fatimah Az-Zahra Palembang, 2025

| Cough with phlegm for ≥ 2 weeks | Coloring results <i>Ziehl-Neelsen</i> | | | | Total | | <i>p-value</i> |
|--------------------------------------|---------------------------------------|------|----------|------|-------|-----|----------------|
| | Positive | | Negative | | N | % | |
| | n | % | n | % | | | |
| Yes | 17 | 70.8 | 7 | 29.2 | 24 | 100 | 0.002 |
| No | 0 | 0.0 | 6 | 100 | 6 | 100 | |
| Total | 17 | 56.7 | 13 | 43.3 | 30 | 100 | |

Based on Table 5.3 above, it can be concluded that of the 30 respondents with phlegmy cough results of ≥ 2 weeks with Ziehl-Neelsen staining, 17 (56.7%) respondents tested positive for tuberculosis. Meanwhile, 13 of the respondents tested negative for tuberculosis (43.3%). The chi-square test results showed a $p\text{-value}$ of 0.002, meaning there is a relationship between phlegmy cough of ≥ 2 weeks and Ziehl-Neelsen staining results.

d. Discussion

1) Univariate Analysis

✓ Incidence of Productive Cough ≥ 2 Weeks

A productive cough lasting ≥ 2 weeks is a major symptom that plays a crucial role in the early detection of tuberculosis. This symptom is used as an initial clinical indicator to



assess the likelihood of pulmonary tuberculosis. Based on the results of this study, most respondents who complained of productive cough ≥ 2 weeks showed positive results in the Acid-Fast Bacilli (AFB) examination, leading to the diagnosis of *Mycobacterium tuberculosis* (MTB) infection. These findings strengthen the evidence that a cough duration of ≥ 2 weeks is closely associated with an increased probability of pulmonary tuberculosis, making it an important guideline in TB screening and case detection in the community.

This finding is consistent with the study conducted by Indra et al. (2020), which reported 62 respondents experiencing productive cough ≥ 2 weeks. Their results indicated that cough duration is an important clinical indicator, as patients with longer cough duration have a higher risk of being confirmed with pulmonary tuberculosis.

However, contradictory findings were reported by L. Lestari et al. (2023), who found that not all patients with cough ≥ 2 weeks were diagnosed with pulmonary tuberculosis. This discrepancy may be due to differences in diagnostic methods (e.g., reliance solely on chest X-ray without AFB confirmation), the presence of other pulmonary diseases such as Chronic Obstructive Pulmonary Disease (COPD), chronic bronchitis, or lung cancer, which also cause prolonged cough, as well as weakened immune systems that result in atypical cough symptoms.

In this study, univariate analysis showed that out of 30 respondents, 24 (80.0%) experienced productive cough ≥ 2 weeks, while 6 respondents (20.0%) experienced productive cough < 2 weeks.

2) Ziehl–Neelsen Staining Results

In this study, Ziehl–Neelsen staining results were categorized into two groups: positive (AFB detected in sputum specimens) and negative (no AFB detected). Univariate analysis revealed that out of 30 respondents, 17 respondents (56.7%) had positive AFB results for tuberculosis, while 13 respondents (43.3%) showed negative results.

This result is consistent with the study by Kassa et al. (2021), which used the Ziehl–Neelsen method and reported that among 520 respondents, 435 (83.65%) tested positive for *Mycobacterium tuberculosis*, while 85 (16.35%) tested negative. The high number of positive cases was associated with previous TB treatment history, nutritional status, and patient education level.

In contrast, Nuryaningsih et al. (2023) reported that among 48 samples, 42 (87.5%) were negative and only 6 (12.5%) were positive. The high rate of negative results was attributed to the limited sensitivity of Ziehl–Neelsen staining, which can only detect TB when bacterial load is high. The quality of sputum significantly affects diagnostic accuracy. Good sputum should originate from the lower respiratory tract, be thick, mucoid or mucopurulent, yellowish to greenish in color, or blood-streaked, with a minimum volume of 3–5 ml. Morning specimens are preferred due to higher bacterial concentration. In contrast, saliva, thin sputum, or small-volume samples often lead to false-negative results.

The Ziehl–Neelsen staining method is a specific microscopic technique used to identify Acid-Fast Bacilli (AFB), including *Mycobacterium tuberculosis*. In TB sputum



examination, positive results are indicated by bright red rods against a blue background under a microscope. The bacterial load can be quantified and reported according to WHO guidelines as +1, +2, or +3, reflecting infection severity.

3) Bivariate Analysis

✓ Distribution of Ziehl–Neelsen Staining Results and Incidence of Productive Cough ≥ 2 Weeks

Based on Table 5.3, among 30 respondents examined at RSUD Siti Fatimah Az-Zahra Palembang, the majority of TB patients experienced productive cough ≥ 2 weeks, totaling 24 respondents (80.0%), while 6 respondents (20.0%) experienced productive cough < 2 weeks. Furthermore, 17 respondents (56.7%) showed positive Ziehl–Neelsen staining results, and 13 respondents (43.3%) showed negative results. These findings indicate a relationship between productive cough ≥ 2 weeks and the occurrence of tuberculosis.

Chi-square statistical analysis showed a significant association between productive cough ≥ 2 weeks and Ziehl–Neelsen staining results in the Microbiology Laboratory of RSUD Siti Fatimah Az-Zahra Palembang in July 2025 ($p < 0.05$).

This result aligns with the study by Indra et al. (2020) using the GeneXpert rapid test, which found that among 61 respondents with productive cough ≥ 2 weeks, 59 respondents (65.5%) were confirmed positive for pulmonary tuberculosis, while 2 respondents (2.2%) were negative. The high positivity rate was attributed to thick, blood-streaked sputum, indicating active TB infection.

Conversely, L. Lestari et al. (2023) reported that among 109 samples, only 18 (16.5%) were positive, while 91 (83.5%) were negative. This discrepancy was due to poor sputum quality mixed with saliva, cough not caused by MTB (e.g., respiratory infections, heavy smoking, allergies, asthma, or viral infections), leading to false-negative results.

A key indication of tuberculosis is a persistent productive cough lasting more than two weeks. To confirm the presence of Acid-Fast Bacilli, laboratory testing using Ziehl–Neelsen staining is employed. Men are more susceptible to pulmonary tuberculosis than women due to higher mobility, smoking prevalence, and alcohol consumption, all of which weaken immune defenses and increase exposure risk.

According to the author's analysis, the TB transmission rate at RSUD Siti Fatimah Az-Zahra Palembang can be observed from the number of sputum samples from patients with productive cough ≥ 2 weeks that tested positive for MTB. These figures reflect the proportion of individuals likely suffering from active TB and capable of transmitting the disease. Patients included individuals from diverse occupations, educational backgrounds (ranging from elementary school to master's degree), and age groups, from children to the elderly.

TB patients are advised to undergo treatment following the DOTS (Directly Observed Treatment, Short-course) program until completion. They should also practice cough etiquette, wear masks, maintain proper home ventilation, adopt healthy lifestyles with



balanced nutrition, adequate rest, and avoid smoking and alcohol. Regular follow-up and continuous education are essential to prevent transmission and improve treatment outcomes.

4. CONCLUSION

Based on the results of this study conducted at RSUD Siti Fatimah Az-Zahra Palembang on the identification of *Mycobacterium tuberculosis* (MTB) in sputum samples of patients with productive cough ≥ 2 weeks using Ziehl–Neelsen staining, the following conclusions can be drawn:

1. This study successfully identified the presence of *Mycobacterium tuberculosis* in patients with productive cough ≥ 2 weeks. Out of 30 respondents, 17 individuals (56.7%) showed positive Ziehl–Neelsen staining results (AFB detected), characterized by bright pink rod-shaped bacteria against a blue background, while 13 individuals (43.3%) showed negative results.
2. Cases of productive cough ≥ 2 weeks were significantly associated with Ziehl–Neelsen staining results, where most positive cases exhibited Acid-Fast Bacilli (AFB). The chi-square test demonstrated a significant relationship ($p < 0.05$), indicating that prolonged cough is significantly associated with pulmonary tuberculosis, although some cases may result from other diseases such as non-TB respiratory infections, chronic bronchitis, or immune disorders.

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