Analysis of the Effectiveness of Mycobacterium Tuberculosis Through Resistant Bacterial Staining and Molecular Rapid Tests

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Article Info	ABSTRACT
	The diagnosis of TB can be confirmed by microscopic examination of smear on the sputum of the patient. However, because this method requires very expensive equipment, it is difficult to implement it in health facilities with simple facilities. Therefore, the Ziehl Neelsen staining method is a fairly simple method of choice and provides high sensitivity and specificity. In the research of Eka Kurniwan, et al. 2016, from the results of examining 40 samples with the Molecular Rapid Test (TCM), 16 people (40%) were found to be positive. The research objective was to know Mycobacterium tuberculosis through sputum examination through Ziehl Nelssen's acid-resistant bacteria staining and Molecular Rapid Test (TCM) of Puskesmas Dempo Palembang based on volume, odor, color and consistency. This type of research is a diagnostic test with a cross sectional study approach. Population and sample of 85 patients. The sampling technique in this study is using the total sampling method. Conclusion There is no effect based on volume, there is an effect based on the smell, color and consistency of sputum, there are differences in the results of Mycobacterium tuberculosis through sputum examination through Ziehl Nelssen's Acid-Resistant Bacteria Staining and Molecular Rapid Test (TCM) at Puskesmas Dempo Palembang.

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1. INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease with a treatment period of 6-8 months, even more than 1 year if the bacteria that causes TB, Mycobacterium tuberculosis, which infects patients, has become immune or resistant to anti-TB drugs.

According to the Ministry of Health of the Republic of Indonesia in 2016, in 2016 the number of new cases of tuberculosis discovered in South Sumatra was 5,674 cases declared BTA (+), including 3,566 men and 2,108 women (Indonesian Health Profile, 2016).

Sputum examination serves to establish a diagnosis, assess the success of treatment and determine the potential for transmission. Examination of sputum for diagnosis is carried out by collecting 3 specimens of sputum which are collected in two consecutive visits in the form of Every Morning-While (Ministry of Health, Republic of Indonesia, 2011).

The diagnosis of TB can be established by microscopic examination of AFB in the patient's sputum. BTA staining can be done using the Tan Thiam Hok, Ziehl Neelsen method, therefore, the Ziehl Neelsen staining method is a choice of 2 methods that are quite simple and provide quite high sensitivity and specificity (WHO, 2017, Indonesian Ministry of Health, 2016).

138

International Journal Scientific and Profesional (IJ-ChiProf)
Vol.1 Issue.2 May 2022, pp: 137-143
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A fast method capable of replacing the culture is polymerase chain amplification (PCR). Tuberculosis can be treated for 9 months or more because the germs grow very slowly and are dormant. With two or more antimicrobials, the possibility of emergence of resistant strains during such a long period of therapy can be minimized.

In December 2010, the World Health Organization (WHO) endorsed Xpert MTB/RIF for use in TB endemic countries. This was done after 18 months of assessing its field effectiveness in TB, MDR-TB and TB/HIV co-infection. This test, and other tests that may be followed, can potentially improve the diagnosis of TB in those who may be missed by traditional tests (Kementrian Kesehatan RI, 2016).

In the study by Eka Kurniwan, et al., 2016, from the results of examining 40 samples using the GeneXpert RT-PCR method, 16 people (40%) were positive and 24 people (60%) were negative and no rifampicin resistance was found. Research by Jasaputra et al (2005) in Eka Kurniawan (2016) also found the same thing, where out of 22 negative smear sputum samples from BP4 Bandung, PCR examination was carried out to obtain positive PCR results in 14 people (63.6%) and negative 8 people (36.4%).

New smear positive cases found in South Sumatra Province are included in the 10 cases with the highest number of diseases in South Sumatra Province. The highest number of suspected TB cases was in Palembang City with a total of 2,601 cases, while the lowest cases for the number of suspected TB were in Empat Lawang District with a total of 299 cases. (South Sumatra Province Health Profile 2019).

Data in the Dempo Health Center laboratory unit in January-December 2018 found 136 suspected tuberculosis sufferers with details of 36 smear positive people who were examined with Zihl Neelsen staining (Report of Dempo Health Center, 2016).

2. RESEARCH METHOD

The type of research used is a diagnostic test with a cross sectional study approach. The research was carried out at the Dempo Health Center in Palembang, October 16 2020 to November 16 2020. The sampling technique in this study was to use the accidental sampling method. Data obtained or collected by researchers directly and analyzed using computer software.

3. RESULTS AND ANALYSIS

Tabel 1 Distribution of the frequency of Tuberculosis sufferers at the Dempo Palembang Health Center

No	Results	Frequency	Percentage
1.	Positive	17	20
2.	Negative	68	80
	Amount	85	100

Based on table 1, 17 patients (20%) were positive and 68 patients (80%) were negative.

Tabel 2 Gender and Age Frequency Distribution of Pulmonary Tuberculosis Patients

No	Umur	Frequency	Percentage
1	Gender: Man Woman	53 32	62,35 37,65
2	Age: ≤ 50 years > 50 years	51 34	60 40

Based on table 2, it was found that 53 patients (62.35%) were male and 32 patients (37.65%) were female. Age of patients 51 patients (60%) with age ≤ 50 years and 34 patients (40%) with age > 50 years.

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Tabel 3 Distribution of sputum macroscopic frequency at the Dempo Palembang Health Center.

No	Macroscopic	Frequency	Percentage
1	Volume:		
	≤ 1cc	48	56,47
	> 1cc	37	43,53
2	Smell:		
	Smell	25	29,41
	No smell	60	70,59
3	Color:		
	Clear	61	71,76
	Cloudy	24	28,24
4	Consistency:		
	Liquid	14	16,47
	Purulent	71	83,53

From table 3, above sputum with volume ≤ 1 cc was 48 (56.47%) and volume > 1 cc was 37 (43.53%). Sputum with odor was 25 (29.41%) while those without odor were 60 (70.59%). Then 61 (71.76%) sputum with clear color while 24 (28.24%) cloudy color. Then 14 (16.47%) sputum with liquid consistency and 71 (83.53%) purulent consistency.

Tabel 4. Distribution of Mycobacterium tuberculosis statistics at the Dempo Health Center Based on Sputum Volume, Odor, Color and Consistency

	Acid Resistant Bacteria (AFB)				P-Value	Molecular Rapid Test (TCM)						P-Value
Volume:	-	%	+	%		-	%	+	%	Total	%	
≤ 1cc	43	89,6	5	10,4		43	89,6	5	10,4	48	100	
> 1cc	25	67,6	12	32,4	0,012	25	67,6	12	32,4	37	100	0,012
Smell:												
Smell	11	44	14	56	0,000	11	44	14	56	25	100	0,000
No smell	57	95	3	5		57	95	3	5	60	100	
Color:												
Clear	56	91,8	5	8,2	0,000	56	91,8	5	8,2	61	100	0,000
Cloudy	12	50	12	50		12	50	12	50	24	100	
Consistency:												
Liquid	11	78,6	3	21,4	0,884	11	78,6	3	21,4	14	100	0,884
Purulent	57	80,3	14	19,7		57	80,3	14	19,7	71	100	

Table 4 shows the results of 48 tuberculosis patients based on volume ≤ 1 cc with the BTA method, positive results of 10.4%, negative results of 89.6% with the TCM method, positive results of 10.4%, negative results of 89.6%. Then the results were also obtained from 37 tuberculosis patients based on volume ≤1cc with the smear method 32.4% positive results and 67.6% negative results with the TCM method 32.4% positive results and 67.6% negative results. The P-value for the smear method examination was 0.012<0.05 and for the TCM method examination it was also 0.012<0.05 so that it can be concluded that there was an influence on Mycobacterium tuberculosis through the Acid-resistant Bacterial Painting Ziehl Nelssen Method and Molecular Rapid Test based on volume.

Based on the smell of 25 tuberculosis sufferers based on smell with the AFB method, positive results were 56%, while 44% negative results were with the TCM method, positive results were 56%, while negative results were 44% The P-value on the smear method examination was 0.000 <0.05 and on the TCM method examination namely 0.000 <0.05 so that it can be concluded that there is an influence of Mycobacterium tuberculosis through the Ziehl Nelssen Method of Acid-resistant Bacteria Painting and the Molecular Rapid Test based on odor.

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140

International Journal Scientific and Profesional (IJ-ChiProf)
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Based on the color of 24 tuberculosis patients with cloudy sputum, the results of the BTA examination method were positive results 50%, negative results 50% with the TCM method, positive results 50%, negative results 50%. While the clear color with the smear method positive results 8.2% negative results 91.8% with the TCM method positive results 5 patients (8.2%) negative results 56 patients (91.8%) P-value on smear method examination is 0.000 <0.05 and on the examination of the TCM method also 0.000 <0.05 so that it can be concluded that there is an influence of Mycobacterium tuberculosis through the Ziehl Nelssen Acid-resistant Bacteria Painting Method and the Molecular Rapid Test based on color.

Based on the consistency of the results of 14 tuberculosis patients based on Liquid Consistency with the AFB method, positive results 21.4%, negative results, 78.6% with the TCM method, positive results, 21.4%, negative results, 78.6% P-value on examination of the AFB method, namely 0.884> 0.05 and on examination of the TCM method, namely 0.884> 0.05. It can be concluded that there is no effect on Mycobacterium tuberculosis through Acid-Fast Bacterial Painting and Molecular Rapid Test for sputum consistency.

Tabel 5. Diagnostic test results of the examination with the BTA method and the TCM method

BTA	TCM		Amount
	Positive	Negative	
Negative	17	0	17
Positive	0	68	68
Amount	17	68	85

Prevalence	**Pr (A)**	**20%**	**12%**	**30,1%**
Sencitivity	+	100%	80,5%	100%
Specificity	-	100%	94,7%	100%
ROC area	(Sen+Spe)/2	1	1	1
Pos predictive value	Pr (A/+)	100%	80,5%	100%
Neg predictive value	Pr (N/-)	100%	94,7%	100%

Based on table 5 above, 85 research subjects responded by taking sputum samples. Sputum examination was carried out using the BTA method and the TCM method. Based on the results of data analysis on the prevalence of suspected TB in the study population of 12%, the sensitivity of smear examination obtained was 80.5%, which means that the ability of smear examination to obtain positive results among people who have suspected TB is 80.5% or among 100 people who suspected TB, 81 (rounded) will be declared positive by smear examination while the rest are negative (false negative). Meanwhile, the specificity of the smear examination was 94.7%, which means that the ability of the smear examination to get negative results among people who are not suspected of having TB is 94.7% or among 100 people who are not suspected of having TB, 95 (rounded up) will be declared negative by the smear examination while the rest were positive (false positives). If the prevalence of suspected TB in a study population is increased to 20% and 30.1%, then a sensitivity of smear examination of 100% is obtained, which means that the ability of smear examination to obtain positive results among people with suspected TB is 100% or among 100 people with suspected TB, then all of them will be declared positive by the BTA examination so that there are no negatives (false negatives). Whereas the specificity of the AFB examination is 100%, which means that the ability of the AFB examination to get negative results is 100% among people who are not suspected of having TB or among 100 people who are not suspected of having TB, all of them will be declared negative by the AFB examination so that no one is positive (false positive).). Thus, it can be concluded that sputum examination using the BTA method for diagnosing TB is the same as using the TCM method.

The positive results in this study were lower, while the negative results were higher compared to research conducted by Diana Susanti (2013) at RSUP. Prof. Dr. R.D Kandou Manado obtained 46% positive BTA examination results, while 54% were negative. This study is in line with the distribution of research conducted at 3 Public Health Centers in Manado City in 2015, which obtained positive results of 2.7% BTA examination. (Ministry of Health, 2018).

In a study by Rukmini and Chatarina (2011) showed that the risk factors for the incidence of pulmonary TB in adults in Indonesia were age, gender, lighting energy, nutritional status, and household

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contact with TB patients. The most dominant risk factor influencing the incidence of adult pulmonary TB is household contact with TB patients.

The research conducted at the Dempo Health Center found positive and negative smear results, not too far apart. This can happen due to many factors. Factors in the quality of the sputum sample can determine whether the result will be correct or will be a false positive or negative. In the study at the Dempo Health Center, there were several samples that were still purulent, which meant that they were not good at interpreting the results of BTA, so that the results could be fake.

This research is in line with research conducted by Zulfiana Amalia which was conducted from June 2016-June 2017, the results obtained from 375 patients as many as 42 patients 11.2% were declared MDR TB, MTB was detected and intermediate resistant as many as 3 patients (0.8%) and MTB was detected and not resistant in 112 patients (29.9%) while MTB was not detected in 200 patients (53.3%) and invalid in 6 patients (1.6%).

The results of this study are not in line with research conducted by Mukhtar Ihsan in 2016 which concluded that out of 19 patients tested using the TCM method, 11 patients (58%) were positive and 8 patients (42%) were negative.

In this study the positive results of AFB were positive after being examined with the TCM method, the results were the same. The results of this study are in line with research conducted at Sri Melati et al at Persahabatan General Hospital in 2005-2007 in which the age of the most dominant tuberculosis sufferers was at the productive age of 25-34 years 35.6%, while the age of the least tuberculosis sufferers was at the age of > 55 years as much as 6.9%.

The same thing happened in Dwi Martono's research (2017) which showed that the incidence of tuberculosis in Pati Regency increased with age. In general, the greatest incidence of tuberculosis occurs in productive age.

According to research conducted by Helper Sahat P Manalu (2010) pulmonary TB disease is most often found in the productive age of 15-50 years.

This research is not in line with research conducted by Maykel Sondak et al at the Paniki Bawah Public Health Center, Tikala Baru and Wonasa Manado. In his research, it was found that the age of tuberculosis sufferers was at the age of 55-79 years 37.5%.

Tuberculosis occurs at a productive age because age is a risk factor for pulmonary TB, where young adults and the elderly have lower immune systems than adults (Achmadi, 2005). A person's age affects the level of knowledge, beliefs, habits, this will affect a person's eating habits/patterns so that it will affect the amount of food intake, variety of food and affect nutritional status. A person's nutritional status will affect a person's level of health (Achmadi U.F., 2005, Notoatmodjo, S. 2007).

The results of this study are in line with research conducted by Rukmini and Chaterina (2011) which stated that the risk factors for pulmonary TB in adults in Indonesia based on gender were more dominant in males 61.3% (117/191). Similar to the research by Nurul Husna Muchtar et al (2015) at Dr. M. Djamil Padang, in this study the distribution of tuberculosis incidence in males was more dominant at 72.3% (47/65), while the incidence of tuberculosis in females was 27.7% (18/65).

The World Health Organization in its Gender In Tuberculosis Research study states that the incidence of pulmonary tuberculosis in women is always lower than that of men. There is still no clarity regarding the reasons for this difference, but it is possible that women have greater adherence to tuberculosis care and treatment.

The Indonesian Ministry of Health reports that more men suffer from pulmonary tuberculosis than women because few women have smoking habits. Smoking can cause the function of the respiratory tract to be disrupted thereby increasing the risk of being infected with tuberculosis.

The recurrence rate of pulmonary TB is higher in men, presumably due to differences in exposure and risk of infection. This is because most men have the habit of smoking, drinking alcohol, and using illegal drugs. In addition, work, weight and average hemoglobin are the things that make men more susceptible to relapse (Jamil, 2009, M. Zainul 2009).

In this study, it was known that differences in the volume of sputum secreted by tuberculosis patients could be affected by the degree of the disease and the stage of the disease. Good sputum expelled from the lungs of tuberculosis sufferers has a volume of 3.5-5 ml. (Ministry of Health RI, 2012) Large amounts of more than 100 ml/24 hours, possibly exceeding 500 ml are found in pulmonary edema, bronchiectasi lung abscess, advanced pulmonary tuberculosis and in abscesses that break through the lungs. Sputum quantity is the amount of sputum produced by measuring the volume of sputum (in ml). Sputum quantity according

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142

International Journal Scientific and Profesional (IJ-ChiProf)
Vol.1 Issue.2 May 2022, pp: 137-143
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to the RI Ministry of Health, 2005: 27, Good: 3-5 ml volume per intake. Not good: the volume per intake is less than 3 ml.

The smell of sputum as one of the conditions for examination must be tested in a fresh state because sputum that is left for a while will rot and lose its distinctive smell. According to the RI Ministry of Health, 2011 a foul odor in fresh sputum can be found in gangrene and pulmonary abscess, in tumors that have necrosis and in empyema that penetrates into the bronchi, if an abscess under the diaphragm penetrates upwards you will get an odor like feces. (Depkes RI, 2011) In this study, the amount of smelly sputum was greater than that of non-smelly sputum. Changes in the smell of sputum are possibly caused by fungus, so the smell becomes strong. (Teguh Budiharjo, 2016).

Based on the color of the sputum, it has a more clear yellow color than a greenish yellow color. This can be caused by the stage of the disease suffered by tuberculosis sufferers. In the quality of sputum with cheese grains, namely small yellow pieces originating from necrotic tissue. Curschman's spiral, which is a rolling yellow thread, pouring bronchi, the casting material is fibrin, the size of which depends on the size of the broncus where it is formed, Ditrich's plug, which is a yellow and white object that is formed in the bronchi or bronchioles. The gray or yellow color is usually caused by pus and epithelial cells. (MOH RI, 2011). Meanwhile, in the yellow-green sputum, it is possible that the process of accumulating pus indicates that treatment with antibiotics can reduce symptoms and is also caused by Neutrophil myeloperoxidase.

In sputum consistency with purulent sputum consistency more than mucopurulent sputum consistency, this is also influenced by the disease and its stage level. Thick yellowish mucopurulent sputum is seen in the early stages of tuberculosis. (Laura A. Talbot, 1997). In purulent sputum caused by degenerated white blood cells. (Allan H. Goroll, 2009).

The results of this study are in line with research conducted by Iqbal Rashid et al (2011) at Dr Goerge Mukhari Hospital South Africa. In his research showed that there is a comparison between sputum volume in sensitivity and specificity, as well as predictive value. A 5 ml sputum volume is consistently superior to a 2 ml sputum volume. Clinically, using a 5 mL sample has a false positive probability of a true negative result almost 50% lower than using a 2 mL sample. It is recommended to collect a sputum sample for microscopic examination of acid-fast bacilli. A minimum of 5 ml of sputum sample is recommended.

In a study, Sharla Badal Faesen et al (2017) revealed a strategy to increase the sensitivity of AFB examination using Xpert MTB/RIF on smear negative smears with suspected tuberculosis. In that study the use of a larger volume (5-10 ml) could optimize the sensitivity of the examination by up to 50%.

The results in this study are in line with the statement according to The Microbiology of Respiratory System Infections (2016) in Science Direct Topics which states that normal sputum does not smell, otherwise foul-smelling sputum indicates a bacterial infection. The same thing was explained by Sylvia (2011) in J Ariyanto (2018), sputum that has a foul odor may be a sign of lung abscess or bronchiectasis.

In this study, the number of patients who were detected positive on the smear examination using the Zeilh Neelsen method and the TCM method based on sputum color had the same number. The results of this study are in line with the research of Attila Altiner, et al. (2009) of 241 patients with acute cough, 28 samples (12%) of them proved to be infected with bacteria. Yellow or green color of sputum showed a significant correlation with bacterial infection (P = 0.014), with a sensitivity of 0.79 (95% CI 0.63-0.94) and a specificity of 0.46 (CI 0.038-0.53). Sputum color plays an important role in the concept of disease (acute cough) for both patients and doctors, but it is not yet clear whether sputum color can diagnose a bacterial infection. (Attila Altiner, et al. 2009).

So that the color of sputum that is good in making the diagnosis of Mycobacterium tuberculosis is a yellow-green color compared to the color of clear yellow sputum, but sputum that does not have a yellow-green or clear yellow color does not mean that it is negative for Mycobacterium tuberculosis because every tuberculosis diagnostic examination has sensitivity and different specificities.

The results of this study are in line with the research of Ari Handoko, et al. 2012, out of 114 mucopurulent sputum specimens subjected to AFB examination, it was found that all mucopurulent samples had positive BTA gradations (1+), (2+) and (3+) with a tendency for the percentage of smear examination results to be almost evenly distributed, namely between 30.7-37.7%. (Ari Handoko, et al. 2013).

So that sputum that has a pure consistency will find Mycobaterium tuberculosis more easily than purulent sputum on AFB examination and especially on Polymerase Chain Reaction (PCR) examination which has better sensitivity and specificity than conventional methods such as AFB examination and culture.

143

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Research on sputum consistency is in line with research conducted by Boehme (2009) in Mukhtar Ikhsan (2016) showing that examination with GeneXpert MTB/RIF to diagnose MDR TB has a high sensitivity of 96.5% and a sensitivity of 96.1% in detecting rifampin resistance. The results showed that the GeneXpert MTB/RIF examination had high validity for MTB examination (Muktar Ikhsan, 2016).

4.   CONCLUSION

Based on the research that has been done, it can be concluded that there is an influence of Mycobacterium tuberculosis through the Ziehl Nelssen Method of Acid-resistant Bacteria Painting and the Molecular Rapid Test based on volume, odor and color. There is no effect on Mycobacterium tuberculosis through Acid-Fast Bacterial Staining and Molecular Rapid Test based on sputum consistency. There is no difference in the results of sputum examination with the BTA method and the TCM method.

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