COMPARISON OF ACID-RESISTANT BACIL EXAMINATION RESULTS IN SPUTUM SUSPECTED OF INTERMEDIATE PULMONARY TUBERCULOSIS ZIEHL-NEELSEN METHOD BY METHOD GENEXPERT IN HOSPITAL TOTO KABILA DISTRICT BONE BOLANGO

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ABSTRACT

This study aims to determine the comparison of the results of acid-resistant bacilli examination in sputum suspected of pulmonary tuberculosis in the two examination methods used to detect Mycobacterium tuberculosis, namely the Ziehl Neelsen method by staining sputum and the GeneXpert method by automatically using the GeneXpert tool on sputum suspected of pulmonary tuberculosis. The method in this study is quantitative with primary data type and sampling technique using purposive sampling with a total of 20 samples. The results showed that both examination methods, namely ziehl neelsen and geneXpert, obtained a significance value (sig.) (2-Tailed) of 0.317 which is more than the value of a (a = 0.05), or 0.317> 0.05 based on the results of statistical analysis, then H_2 is accepted and Ho is rejected.

Keywords: BTA, Suspects, Pulmonary tuberculosis, *Ziehl-neelsen, GeneXpert.*

INTRODUCTION

Mycobacterium Tuberculosis is a bacterium that causes tuberculosis which is transmitted through saliva droplets from the patient when the TB patient coughs or sneezes. The Mycobacterium tuberculosis bacillus bacteria then accumulates in the free air which then infects other people through droplet infection. Inhaled bacteria will enter the respiratory tract and attach to the surface of the lung alveolus until it then invades other patients with pulmonary tuberculosis disease. After infecting the lungs, tuberculosis bacteria will stimulate an increase in secretions in the form of patient secretions (Firdhani, 2020) [14].

Tuberculosis can affect any organ of the human body, although most tuberculosis affects the lungs, but the incidence of extra-pulmonary or TB disease outside the lungs is reported to reach 5 to 30%. If left untreated or incompletely treated, tuberculosis can lead to dangerous complications and even death. Although the number of deaths from tuberculosis decreased by 22% between 2000-2015, tuberculosis still ranked as the 10th leading cause of death worldwide in 2016. Therefore, TB is still a top priority in the world and is one of the goals in the SDGs (Sustainability Development Goals) (Pramasari, 2019) [4]

According to the World Health Organization (WHO), the number of cases between 2000 and 2015 decreased by 22%; however, TB still ranked as the 10th leading cause of death in the world in 2016 (MOH, 2018).

According to the Indonesian Ministry of Health (2018), the number of TB cases in Indonesia was 420,994 cases in 2017

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and according to Riskesdas data (2018) in the top three were found in Papua Province as many as 0.77% of TB sufferers, followed by Banten Province as much as 0.76%, and in West Java Province which is 0.63%. In TB cases that occurred in 2018 in Gorontalo province as many as 0.42% of TB patients.

According to the Gorontalo Provincial Health Office (2019), the prevalence rate of TB cases varies from Bone Bolango district 93.42%, Gorontalo district 85.57%, Gorontalo city 72.48%, North Gorontalo district 71.70%. Pohuwato district 67.32%, and Boalemo district 55.43%.

According to data contained in the Tuberculosis Information System (SITB) of Toto Kabila Hospital, Pohuwato District in 2023 from June to August, there were 329 suspected TB patients, including 254 negative and 75 positive patients.

Rapid and accurate diagnostic methods are essential for the diagnosis and control of pulmonary TB in Indonesia. Ministry of Health Regulation No. 67/2016 on Tuberculosis Control recommends three types of bacteriological examinations to diagnose TB, namely microscopic examination with Ziehl Neelsen (ZN) staining, Molecular Rapid Test (TCM) with GeneXpert MTB/RIF, and culture. The examination is carried out in accordance with the availability of facilities at health service facilities (fasyankes). If the bacteriological examination is negative, a thorax X-ray examination is performed. (Indonesian Ministry of Health, 2016).

Research by Munir et al. (2015) showed that the positivity, sensitivity and specificity of ZN staining in lung specimens to diagnose pulmonary TB were 67.5%, 77.7% and 91.4%, while the positivity, sensitivity and specificity of GeneXpert MTB/RIF were 77.4%, 90.1% and 98.3%.

Examination with ZN staining also cannot distinguish between drug-sensitive MTB and drug-resistant MTB and cannot distinguish between MTB and nontuberculous mycobacteria (NTM). (Ramadhan, 2018)

Some limitations of GeneXpert MTB/RIF are that it cannot monitor the success of TB treatment, cannot distinguish between viable MTB and non-viable MTB, requires a cooling machine to maintain temperatures between 15-25° C, and requires electricity throughout the reaction so it is not suitable in places with limited electricity. (Ministry of Health, 2017)

Acid-resistant bacteria are a group of bacteria that have special properties resistant to decolorization or color fading with alcohol acids so they are classified as gram-positive bacteria. This group of bacteria has a rod or bacillus shape so it is also known as Acid Resistant Bacillus (BTA). (Ayu, 2022)

Tuberculosis (TB) is an infectious disease that primarily affects the lung parenchyma. Tuberculosis can be transmitted to other parts of the body, including the meninges, kidneys, bones, and lymph nodes. The main infectious agent of TB is Mycobacterium tuberculosis. Mycobacterium tuberculosis is an acid-resistant aerobic rod bacterium that grows slowly and is sensitive to heat and ultraviolet light (Pramasari, 2019).

Mycobacterium Tuberculosis is a straight or slightly curved rod, without spores and not encapsulated. This bacterium measures 0.3-0.6um wide and 1-4µm long. (Iriranti et al, 2016)

In the early stages of Pulmonary TB disease, there are no specific signs and symptoms. However, along with the course of the disease, the lung tissue will be damaged, which can increase sputum

production as indicated by the client's frequent coughing as a form of compensation for sputum discharge. In addition, clients can feel tired, weak, sweat at night and experience weight loss. (Pramasari, 2019)

Mycobacterium Tuberculosis is transmitted through the air, when people with active pulmonary tuberculosis (BTA positive and X-ray positive) cough, sneeze, shout or sing, the bacteria will be carried out of the lungs into the air. These bacteria will be in liquid bubbles called droplet nuclei. Transmission of tuberculosis occurs when a person inhales the droplet nuclei which will pass through the mouth/nose, upper respiratory tract, bronchi and then into the alveolus. After the tubercle bacillus reaches the lung tissue, the bacteria will begin to multiply and will gradually spread to the lymph nodes. (Azizah, 2018).

According to Diana Putri (2020), there are several diagnoses of tuberculosis, namely:

1. Clinical Examination

   Clinical examination is by looking at complaints or symptoms from the patient, as well as the physical condition of the patient. It can also be proven by a tuberculin test. The time between infection and a positive tuberculin reaction is 4-5 weeks and the incubation period from infection to illness is 6 months.
2. Radiology Examination

   Radiological examination, namely X-rays of the lungs, can strengthen the diagnosis of BTA positive. The results of X-rays of the lungs infected with BTA positive pulmonary tuberculosis disease in the picture show "spots" or white spots on the lungs. X-rays are done in two positions, namely from the front and from the side, lung X-rays are done at the beginning and at the end of treatment to monitor the success of treatment after 2 months and 6 months of treatment.
3. Laboratory Examination

   Laboratory examination is the examination of tuberculosis using a microscope. A person is declared infectious pulmonary tuberculosis based on symptoms and examination of sputum cough containing Mycobacterium Tuberculosis germs in 3 times of sputum cough examination.

Ecpectoratorian or commonly referred to as sputum is mucus and other material carried from the lungs, bronchi and trachea that may be coughed up and regurgitated or swallowed. Unlike saliva, sputum is thicker and does not have foam bubbles on it (Azizah, 2016).

Sputum quality is assessed based on volume, color and viscosity, which is at least 3 ml, with a yellowish color (purulent), and viscous like phlegm or good viscosity. Those that are slightly thick and mixed with secretions are called mucopurulent and those with watery viscosity or saliva/secret-like are called poor quality. At least one sample out of three sputum samples (during, morning, during) should be of good quality. (Nani, 2020)

Sputum examination serves to establish a diagnosis, assess treatment success and determine transmission potential. Sputum examination for diagnosis is carried out by collecting 3 sputum specimens collected on two consecutive days of visits in the form of Time-Morning-Time. (Herry, 2022)

a. Sputum collection technique

   According to the Indonesian Ministry of Health (2017) the sputum collection procedure is as follows:

   1) Provide a minimum 4-threaded, new, clean, wide-mouthed sputum pot (± 5cm diameter).
   2) Write the patient's name and sputum specimen identification

number on the wall of the sputum pot according to the naming rules of the national guidelines. Do not write the patient's identity on the lid of the sputum pot.

3) Collection of sputum specimens should be done in a sputum booth located in an open space, in direct sunlight, with a washbasin, hand soap, infectious waste bins, tissues, and not heavily trafficked. If wearing dentures, remove them before gargling.
4) Gargle with drinking water before expelling phlegm.
5) Take 2-3 deep breaths and exhale forcefully each time.
6) Place the opened phlegm pot close to the mouth.
7) Cough hard from inside the chest and expel the phlegm into the pot. Close the sputum pot immediately and tightly. Avoid spilling or soiling the outside of the container and then tighten the lid on the collection container. TCM examination requires a sputum volume of at least 1 ml.
8) Clean your mouth with a tissue and dispose of the tissue in the closed trash can provided and then wash your hands with soap and antiseptic.

Ziehl-Neelsen staining is a differential stain which means that it uses more than one stain for acid-resistant bacteria. Acid-resistant bacteria have thick cell walls consisting of a layer of wax and mycolic fatty acids. So that even though it is washed with 5% sulfuric acid, acid-resistant bacteria will still bind the dye from carbolic fuksin. (Ira, 2019)

The advantages of the microscopic examination method with Ziehl-Neelsen (ZN) staining are still the first in detecting early TB infection. Because this technique is considered easier to perform, efficient, inexpensive and also has high specificity to detect acid-resistant bacteria. In addition, this method can also be used in all laboratory units, including the simplest laboratories (Khariri, 2020).

The disadvantages of this method are that there are several factors that can affect the sensitivity of sputum microscopic examination with Ziehl-Neelsen staining, such as the prevalence of tuberculosis, the quality, number of specimens, coloring methods and the ability and laboratory personnel who perform the examination (Khariri, 2020).

Basically, the principle of BTA staining is to utilize heat and phenol in order to penetrate the fat or wax layer in the cell wall so that carbolic fuccin or basic dyes can penetrate the cell fat layer. After being stained with carbolic fuxin and rinsed with running water, the exposed fat layer will be closed again due to cooling when rinsed so that when poured with acid alcohol the red dye from carbolic fuxin will not be released. Finally, when staining with methylene blue, BTA will not absorb the dye while bacteria that are not acid-resistant bacteria will absorb the blue color of methylene blue. (Nazarudin, 2015)

GeneXpert MTB/RIF is an automated Nucleic Acid Amplification Test (NAAT) based cartridge test used to detect TB and rifampicin-resistant cases and provides results in less than 2 hours. (Izer, 2019)

The TCM is a nested real-time PCR (Polymerase Chain Reaction) based molecular detection method for TB diagnosis. The PCR primers used are capable of amplifying approximately 81 bp of the core region of the MTB complex rpoB gene, while the probe is designed to distinguish wild type sequences and mutations in the core region associated with resistance to rifampicin. The screening was performed with the GeneXpert tool, which uses an automated system that integrates the

processes of specimen purification, nucleic acid amplification and target sequence detection. The system consists of a GeneXpert device, computer and software. (Ministry of Health, 2017)

RESEARCH METHODS

The approach used in this research is a quantitative approach with analytical research type. This research was conducted from October 26 to October 06, 2023 at Toto Kabila Hospital. The population in this study were all suspects of pulmonary TB at Toto Kabila Hospital, Bone Bolango Regency from June to August with a total of 75 people and the number of samples obtained based on sample criteria was 20 people with purposive sampling technique.

The tools and materials used in this study are sample pots, microscopes, GeneXpert tools, glass objects, Bunsen lamps, Bio Safety Cabinet (BSC), pipettes, geneXpert catridge, sticks, tweezers, staining racks, sputum samples, Ziehl-Neelsen staining reagents, immersion oil.

RESEARCH RESULT

3.1 Research Result

Table 4.1 Research Results of Acid Resistant Bacillus (BTA) Examination in Sputum Suspected of Pulmonary Tuberculosis Between the Ziehl-Neelsen Method and the GeneXpert Method at Toto Kabila Hospital, Bone Bolango Regency.

Sample code	Gender	Age	BTA Test Result	
			ZN Painting	***Genexpert* Method**
A	L	75	Negatif	Not Detected
B	L	31	Negatif	Not Detected
C	L	49	Positif 1	Detected Medium
D	P	54	Negatif	Not Detected
E	L	30	Positif 2	Detected High
F	P	50	Negatif	Not Detected
G	L	63	Negatif	Not Detected
H	L	72	Negatif	Not Detected
I	P	61	Negatif	Not Detected
J	L	19	Negatif	Not Detected
K	P	17	Negatif	Not Detected
L	L	18	Negatif	Not Detected
M	P	61	Negatif	Not Detected
N	L	20	Negatif	Not Detected
O	P	57	Negatif	Not Detected
P	P	67	Negatif	Not Detected
Q	L	75	Negatif	Not Detected
R	P	18	Negatif	Not Detected
S	L	49	Negatif	Not Detected
T	L	45	Positif 1	Detected High

Source: Personal research data, 2023

Based on table 4.1 above, it can be obtained that the number of samples in this study was 20, with the results of BTA examination on ZN painting there were 3 (three) positive samples (sample codes C, D, and T), as well as the geneXpert method which had 3 (three) positive samples which were also the same respondents (sample codes C, D, and T).

Univariate Analysis

Table 4.2. Distribution of Respondents Based on Gender

Gender	**Frequency**	**Percentage(%)**

Male	12	60.0
Female	8	40.0
Total	**20**	**100**

(Source: Personal research data, 2023)

Table 4.2 above shows that of the 20 respondents studied in this study, the majority were male, namely 12 people (60%), and 8 people (40%) were female.

Table 4.3. Distribution of Respondents by Age

Age	**Frequency**	**Percentage (%)**
10-20 years	5	25.0
21-30 years	1	5.0
31-40 years	1	5.0
41-50 years	3	15.0
51-60 years	3	15.0
Di atas 60 years	7	35.0
Total	**20**	**100**

(Source: Personal research data, 2023)

Table 4.3 above shows that of the 20 respondents suspected of Pulmonary TB in this study, the majority were over 60 years old, namely 7 people (35%), then 10-20 years old as many as 5 people (25%), respondents aged 41-50 years and 51-60 years each as many as 3 people (15%), while respondents aged 21-30 years and 31-40 years each only 1 person (5%).

Bivariate Analysis

Table 4.7. Normality Test of Modified and Unmodified BTA Examination.

BTA Test Rseult	**NPar Tests (*One-Sample Kolmogorov-Smirnov Test)***		
	Statistic	*N*	*Sig*
***Ziehl-Neelsen* (ZN)**	.438	20	.000

(Source: Personal research data, 2023)

Based on table 4.7 about the results of the NPar Tests normality test with the One- Sample Kolmogorov-Smirnov Test for the results of BTA examination between the ZN staining method and the GeneXpert method, it can be seen that the value of asymp sig. (significance) is 0.000 which is smaller than the value a = 0.05, or 0.000 <0.05.

Based on the normality test decision making with NPar Tests that if the asymp sig value. (siginfikansı) is smaller than the a (0.05) value or sig <0.05, then the data is declared not normally distributed, while if the asymp sig. (siginfikansi) is greater than the value of a (0.05) or sig> 0.05, then the data is declared not normally distributed. So it can be concluded that the data from the BTA examination results with the ZN staining method and the geneXpert method in this study are not normally distributed.

Because the data in this study were not normally distributed, the bivariate test was not continued with the parametric method using the independent sample t-test, but continued with the non-parametric method using the Wilcoxon Signed Rank Test, which is a non-parametric test whose purpose is to determine whether there is a statistically significant difference between two groups of paired data with ordinal or interval scales but with abnormal distribution in the normality test results.

Table 4.8. Non-parametric analysis of BTA test results between Ziehl-Neelsen and GeneXpert.

BTA Test Resukt	Significant (*2-Tailed*)	Significance Level	Information
***Ziehl-Neelsen* (ZN)**	0.317	0,05	There is no significant difference
***GeneXpert* Method**			

(Source: Personal research data, 2023)

Table 4.8 above shows the results of the non-parametric test analysis using the Wil coxon Signed Rank Test method. The results of this data processing show that in the results of BTA examination in

suspected pulmonary tuberculosis between the ZN staining method and the GeneXpert method, the significance value (sig.) (2-Tailed) is 0.317 which is greater than the a value (a = 0.05), or 0.317>0.05. So, referring to the conclusion making in the Wilcoxon Signed Rank Test, it can be concluded that the results of BTA examination in pulmonary tuberculosis suspects between the ZN staining method and the GeneXpert method have no significant difference, in other words, the null hypothesis (Ho) is rejected and the alternative hypothesis (H_2) is rejected.

DISCUSSION

The absence of differences in results between the two methods, namely the ZN staining method and the GeneXpert method in this study, according to the researcher is due to the advantages of the two methods. In ZN staining, in accordance with the theory mentioned in Ayu (2022), that acid-resistant bacteria (BTA) are a group of bacteria that have special properties that are resistant to decolorization or color fading with alcohol acids so that BTA is classified as gram-positive bacteria. The acid-resistant nature of these bacteria is because BTA has Acid-fast characters, namely the condition of thick cell walls due to the presence of high levels of peptidoglycan and lipid content so that cells become waxy, hydrophobic and difficult to color. However, with the presence of several compositions in the ZN dye in the form of a primary dye, namely 1% carbolic fucin, which functions to provide contrasting colors to all cells and open the cell walls of Mycobacterium Tuberculosis which contains a high concentration of fat layers to facilitate the absorption of dyes, the presence of Mycobacterium Tuberculosis can be found in sputum preparations (Widya, 2021).

This staining technique was first initiated by two German doctors, Franz Ziehl (1859- 1926), who was a bacteriologist and Friedrich Neelsen (1854-1894), who was a pathologist. ZN staining is a special bacterial dye used for acid-resistant organisms/bacteria, especially Mycobacteria.

Anam and Rahmawati (2022), added that ZN staining is differential staining, namely staining that uses more than one type of dye and can distinguish acid-resistant bacteria from bacteria that are not acid-resistant. The ZN staining technique has advantages in BTA examination, namely the presence of a bright blue background, so that the red bacilli appear clearly and the reagents are affordable and easy to obtain. In addition, this staining technique can be done quickly, easily, accurately and does not require expensive costs. However, in practice, the ZN staining technique also has disadvantages, for example in BTA painting not only TB bacteria are able to absorb color, but there are also other bacteria that are able to absorb color like TB bacteria, called Mycobacteria Other Than TB (MOTT). Examples of MOTT are M. kansasi, M. avium, M. intracellular, M scrofulaceum, M. malmacese, and M. xenopi. This leads to false positives, resulting in a less rapid diagnosis of the patient's disease.

The factors that can cause this, according to researchers, are due to the advantages of the GeneXpert method and the weaknesses of the ZN staining method in detecting the presence of BTA in sputum. This is in accordance with the statement of the Ministry of Health of the Republic of Indonesia (2017), in the book "Technical Guidelines for TB Examination Using Molecular Rapid Tests", which states that the difference in microscopic and GeneXpert results occurs because microscopic examination

in sputum must contain at least 5000 germs / ml of sputum to get a positive result, the amount of mucus tissue will increase the sample volume, making it less likely to be able to take samples containing M. tuberculosis germs. Tuberculosis, while the GeneXpert examination with a molecular detection method based on nested real-time PCR is able to detect complex MTB DNA qualitatively, making it possible that the BTA microscopic examination is not found but can be detected in the GeneXpert examination, because GeneXpert is able to detect MTB in destroyed form.

The results of this study are in line with the results of research found by Relasiskawati (2020), and Husna and Dewi (2020). In Relasiskawati's research (2020), it was concluded that the suitability test calculated by Kappa cohen was 0.88, which means that microscopic examination with TCM (GeneXpert) on TB suspect research subjects has a very good level of suitability. Meanwhile, the results of Husna and Dewi's research (2020) showed that there was no significant difference in results between the decontamination method (microscopy with Ziehl-Neelsen/ZN painting) and the molecular rapid test method at the 95% level of significance (a = 0.05), thus concluding that both methods have the same diagnostic ability in identifying Mycobacterium Tuberculosis and can be used according to the needs, costs and laboratory facilities.

CLOSING

Conclusion

Based on the results of the research and data analysis that has been carried out, it can be concluded as follows:

1. The results of the examination of acid-resistant bacilli in sputum suspected of pulmonary tuberculosis using the Ziehl-Neelsen method at Toto Kabila Hospital, Bone Bolango Regency were Negative as many as 17 samples (85%), Positive results as many as 3 samples (15%) with details: Positive result 1 (Post 1) as many as 2 samples (10%), and Positive result 2 (Post 2) only 1 sample (5%).
2. The results of acid-resistant bacilli examination in sputum suspected of pulmonary tuberculosis using the GeneXpert method at Toto Kabila Hospital, Bone Bolango Regency were Negative as many as 17 samples (85%), Positive results were 3 samples (15%) with details: 1 sample (5%) TCM Detected Medium result, and 2 samples (10%) TCM Detected High result.
3. There is no significant difference in BTA results between the Ziehl-Neelsen method and the geneXpert method at Toto Kabila Hospital, Bone Bolango Regency, with the acquisition of a significance value (sig.) (2-Tailed) of 0.317 which is greater than the a value (a = 0.05), or 0.317> 0.05, then Ha rejected Ho accepted.

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