THE EFFECT OF THE DURATION OF MORNING PHELGM STORAGE AT ROOM TEMPERATURE ON THE NUMBER OF ACID RESISTANT BACTERIA

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ABSTRACT

Tuberculosis is an infection of the bacterium mycobacterium tuberculosis which attacks and damages the tissues of the human body. The bacteria can be transmitted thourgh tuberculosis airways usually attacking the lugns but usually also speards the bones, lymph nodes, central nervous system, heart and other organs. Phlegm examination directly relives many weaknesses that is a lot of mucus and tissue that will increase the volume of the sample so that it will reduce the possiblty of getting microbacterium tuberculosis. Therefore, to improve the effevtiveness of sputum microscopic examination, a sample of 4% NaOH sample can be processed so that BTA will be collected in smaller volumes and it is more likely to obtain sample containing germs. This study was conducted with 3 treatmeants, namely morning phlegm which was examined immediately, morning phlegm delayed 3 hours, and morning phlegm delayed 6 hours then decontaminated and examined under a microscope to see how much influence. Based on the analysisof Variance (*ANOVA)* test, it was found that significant value of the *ANOVA test of* 1,000>0,05 did not have a significant effect between the phlegm examined and immediately delayed 3 hours and delayed 6 hours**.** The researchers concluded that the length of the morning phelm storage at room temperature was examine immediately, delayed 3 hours and delayed 6 hours had no effect on the number of acid-resistant bacteria (BTA) (ρ=1,000) thus te alternative hypothesis in this study was rejected. But even though there was no significant effect on the duration of storing phlegm in the morning, the researchers still segested that they still use morning phlegm which was immediately examined in order to still get the quality of phlegm and good examination result.

Keyword: Storage time, Decontamination Of BTA, Examination Sampel

INTRODUCTION

Background

Tuberculosis (TB) is an infection of the bacterium mycobacterium tuberculosis which attacks and damages human body tissues. These bacteria can be transmitted through the airways. TB usually attacks the lungs, but usually also spreads to bones, lymph nodes, central nervous system, heart and other organs (Lika Aprilla Samiadi 2017).

Tuberculosis (TB) is one of the 10 deadly diseases in the World. People affected by this disease are usually characterized by symptoms of persistent cough, accompanied by weight loss, sometimes shortness of breath, and night sweats even when not doing activities (Aprila Puji 2018)

It is estimated that there were 8.6 million TB cases in 2012 of which 1.1 million people (13%) were HIV positive patients. About 75% of these patients are in Africa, in 2012 there were an estimated 450,000 people suffering from MDR TB and 170,000 of them died. In 2012 it was estimated that the proportion of pediatric TB cases among all TB cases globally reached 6% or 530,000 pediatric TB patients per year, or about 8% of the total deaths caused by TB (WHO,2013).

In 2016, there were 274 deaths per day in Indonesia, in the same year, TB cases reached 1,020,000 people. That number makes Indonesia ranked second most tuberculosis cases in the world after India. Then followed by China, the Philippines, Pakistan, Nigeria and South Africa (WHO 2017).

There are an estimated 1,020,000 TB cases in Indonesia, but only 420,000 have been reported to the Ministry of Health. That number beat China in third place which has around 1.4 billion inhabitants. Only one country has worse TB cases than Indonesia, namely India, which has a population of 1.3 billion. (WHO,2017)

Because of the many cases, a bacteriological examination was conducted aimed at identifying the mycobacterium tuberculosis bacteria in the patient's sputum. Currently there are several types that are used for tuberculosis (TB) examination, one of which is microscopic sputum examination.

Direct sputum examination without processing has been done at the initial examination site, but this method still has many weaknesses, namely, a lot of mucus and tissue that increase the volume of the sample, so that it will reduce the possibility to be able to take samples containing mycobacterium tuberculosis. Therefore, to increase the effectiveness of sputum microscopic examination a homogenization method can be processed with 4% NaOH so that smear will be collected in smaller

volumes and will increase the likelihood of taking samples containing germs (Darmawati,2013).

Sputum examination to establish the diagnosis is done by collecting 3 sputum specimens collected in two consecutive days of visits in the form when (S) the first time the patient comes, morning (P) the next day when the patient comes again carrying sputum morning (first phlegm after waking up), when (S) when the patient arrives at the laboratory the patient is asked to expel his phlegm again (SPS). Before making sputum preparations, laboratory workers must physically examine the thick, purplish-green-colored, sometimes blood spots, so that the preparations become more qualified. (Budiharjo,2016)

BTA examination must use a good sputum sample in accordance with the standard so as to produce a good diagnosis so that false positives or false negatives occur and other undesirable things. Usually a good sample to make an examination is a sample of morning sputum that is released by patients when they wake up in the morning because phlegm in the morning is sputum that still has a lot of germs. The sample is produced before eating or drinking and before brushing your teeth, but has been gargled with water to clean the remaining food in the mouth that is left behind. Sputum that meets the requirements must be absolutely correct from the trachea and bronchi, not saliva. (Fujiki A, 2015)

BTA examination is carried out by making preparations with samples (SPS) that have been collected. Based on the above theory the quality of the sample greatly affects the amount of acid-resistant bacilli, in this case, researchers do not yet know how the amount of acid-resistant bacilli in samples that are subject to inspection delays, because usually in the workplace sample delays occur due to the large number of samples handled by laboratory personnel, so often Next time the inspection is postponed. In this case, the researcher is interested in conducting a study entitled '' the effect of the duration of sputum morning storage at room temperature on the number of acid-resistant bacteria (BTA) ''

Problem Formulation

Based on the problem above, the formulation of the problem is whether there is an effect of long sputum storage at room temperature on the number of acid resistant bacteria (BTA)?

Objectives

1. General purpose
   It is known that the effect of long sputum morning storage at room temperature on the number of acid-resistant bacteria (BTA)
2. Specific objectives
   Determined effect of duration of storage of morning phlegm at room temperature on the number of acid resistant bacteria (BTA)

Research Benefit

1. For researchers
   To add more in-depth knowledge about smear positive pulmonary TB sufferers
2. For academics
   As a reference material and information for students and is expected to be continued for further researchers.
3. For the Community
   Provide information to the public about the importance of the BTA test

Conceptual framework

Effect of duration of storage of morning phlegm at room temperature on the amount of smear microscopic to see the change in the number of acid-resistant bacillus bacteria in tuberculosis preparations. To conduct the laboratory examination, an examination is carried out, that is, the sample that has been brought by the patient in the morning is a sputum sample which is immediately examined at that time. Then the rest of the examination sample is stored at room temperature and allowed to stand for 3 hours then the rest of the same sample is left to stand for 6 hours, each of which is decontaminated using 4% NaOH before making preparations, the goal is to get more smear ... Sputum is a liquid which is obtained from the throat which is completely removed from the trachea. For sputum examination itself there are several factors that can affect the results of the examination, namely, the type of sputum itself, if the sputum produced by a patient is not in accordance with the standards, it will affect the results of the examination at the time of reading. The number of bacteria to determine the BTA gradations, a laboratory officer looks at the number of bacteria

present in the preparations made from the sputum. If the quality of sputum is not good then the number of bacteria can be reduced. Furthermore, laboratory staff is a person who examines sputum samples, if the laboratory staff does not inform the patient of a good sputum sample, it will be difficult to carry out the analytical and post-analytical stages and cannot be denied. Therefore, the researcher wants to see the difference in the amount of acid-resistant bacilli (BTA) in the samples that are directly examined and samples that are postponed.

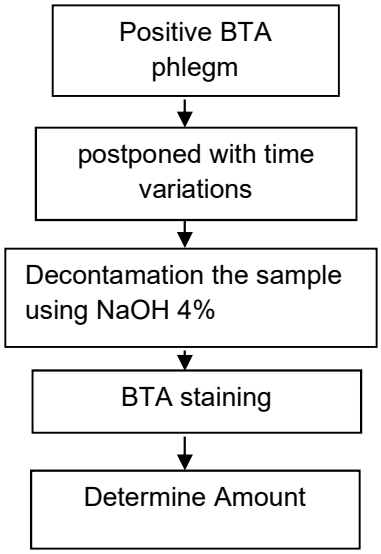

Figure 1: Conceptual Framework

RESEARCH METHODS

Types of research

This type of research is quasi-experimental research design to determine how much influence the duration of storage of morning phlegm at room temperature on the number of acid-resistant bacteria (AFB)

Place and Time of Research

1. Place of research
   The research site was conducted at the Microbiology Laboratory, Makassar's lung health center
2. Research time
   The research time is conducted in January to April 2019

Population and Sample

1. Population
   Phlegm in patients with positive pulmonary tuberculosis
2. Sample
   The sample used in this study was phlegm positive for pulmonary *tuberculosis* patients .
3. Sampling Technique
   Samples were taken by purposive sampling with certain considerations as in this study that determines a positive sample of pulmonary TB
4. The sample size in this study was 9

   The sample size is calculated based on the Federer test using the formula:
   $(t - 1)\ (n - 1) \geq 15$
   $(3 - 1)\ (n - 1) \geq 15$
   $2n - 2 \geq 15$
   $2n \geq 17$
   $n \geq 8{,}5$
   $n \approx 9$
   Description:
   n: number of samples
   t: number of treatments

5. Sample Criteria
   a. Inclusion criteria
      Inclusion criteria in this study are: patients with smear sputum positive results, patients who seek treatment at the Makassar Community Lung Health Center
   b. Exclusion criteria
      The exclusion criteria in this study are: someone with smear sputum negative results, someone who does not seek treatment at the Makassar Community Lung Health Center

Research Variable

1. Independent variable
   Duration of sputum storage
2. Dependent variable
   Amount of acid resistant bacteria (BTA

Operational Definition

1. postponement of sputum examination with 3 treatments, morning phlegm immediately, delay 3 hours and delay 6 hours
2. Acid-resistant bacteria are bacteria which in ziehl-neelsen (ZN) painting still bind the first color, do not fade by alcoholic acid, so they are unable to bind the second color.
3. Acid-resistant bacteria when observed under a microscope appear red with a light blue base color

Research Procedure

1. Pre analytic
   a. Patient preparation
      Patients are given an explanation of the correct cough to get phlegm (sputum **)** thick and purified.
   b. Sample preparation
      Sputum sample is collected in a wide-mouthed pot, holds 6 cm or more with a threaded lid, does not break easily and does not leak.
   c. Good sputum criteria
      Phlegm is good for examination is thick phlegm usually yellow (purelen) **,** greenish yellow (muko purelen) with a volume of 3-5 ml.
   d. Principle
      Acid-resistant bacterial walls have a coating of wax and fat that is difficult to penetrate paint, with the effect of phenol and heating, the waxy layer and fat can be penetrated by carbol fuchsin

Prepare tools and ingredients:

a. Tools: microscope, staining rack, slide / glass object, spiritus lamp, skewer, turbo mixer, centrifuge, pipette, centrifuge tube
b. Ingredients: phlegm
c. Reagents: carbol fuchsin 0.3%, Hcl-alcohol 3%, methylene blue 0.3%, auadest, and oil emersion, NaOH 4%, phosphate buffer saline (PBS)

2. Analytic
   Method: microscopic
   Work procedures:
   Ways of making sputum decontamination:
   a. Sputum sample dilution using 4% NaOH with a ratio of 1: 1 (1 ml sample: 1 ml NaOH)
   b. Homogeneous using a turbo mixer, divortex for a few seconds, confirmed that the specimen was completely mixed
   c. Leave for 15 minutes at room temperature
   d. Then phosphate buffer saline (PBS) ph of 6.8 to 7.0 is added to the 45 ml volume, after that
   e. Centrifug the specimen at a speed of 3000 G for 15 minutes
   f. The supernatant is removed slowly and the precipitate is added with 1 ml PBS 6 , 8 -7.0. Furthermore, the decontamination results are prepared with the help of a pipette of 100 µl,

   How it works making preparations:
   a. Made from the examination material, flatten on a glass object with a size of approximately 2 × 3 cm, the length of the preparation 3 cm and width 2 cm, the preparation should not be too thick or thin, the preparation is then put on a plate then the BTA is applied
   b. How it works staining: placed and then dropped the preparation with carbol fuchsin until it covers the entire surface of the glass object, with low heat, heat the preparation from below (may not boil) this, done 3 times in 5 minutes, rinsed the preparation with running water, then dissolve it with Hcl-Alcohol 3% until all the dyes come out, rinse with water, then drop with methylene blue solution for 30 seconds. Rinse with running water, the preparation is dried on a drying rack in the open air (not to be exposed to direct sun)
   c. How to read the preparations: drop emersion oil over the sputum smear, check using a 10 × ocular lens and 100 × objective, read from the right end on the longest horizontal line in 100 µl of phlegm deposits

3. Post analytic
   Count the number of acid-resistant bacilli (BTA) bacteria per 100 µl of phlegm precipitate diluted with phosphate buffer saline . (PBS)

Data Analysis

To see the effect of sputum storage in the morning in this BTA examination used an *analysis of variance (ANOVA)* test

Operational Framework

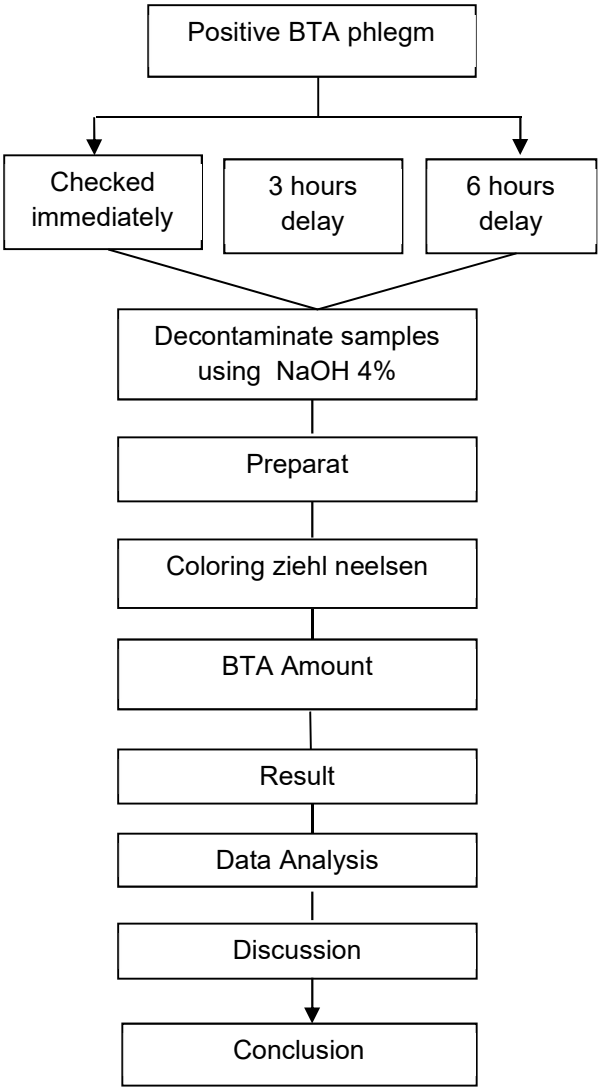

Figure 2. Operational framework

RESULT AND DISCUSSION

Result

This research was carried out on March 25 -08 April 2019 at the Microbiology Laboratory of the Makassar Public Lung Health Center located on Jalan AP Pettarani No. 43, Masale, Panakukkang sub-district, Makassar City, South Sulawesi 90231. The location of the research conducted is easily accessible so that it can facilitate research. The location is only 3 , 6 kilometers from the Makassar Health Polytechnic campus Health Analyst Department.

The purpose of this research is to find out the effect of long sputum storage at room temperature on the number of acid-resistant bacteria.

There were 9 samples of sputum in patients with pulmonary tuberculosis, where in 1 sample 3 treatments were examined which had previously been carried out the process of sputum decontamination. Sputum decontamination process aims to kill or eliminate germs other than microbacteria. In addition, the decontamination process is carried out so that germs scattered in the examination material will gather or be concentrated, making it easier to obtain mycobacteria in large quantities.

From the examination results, the following results are obtained:

1. **Description of Respondent Characteristics**

Table 1
Distribution of Respondents by Age

No	Age (years old)	frequency	Percent (%)
1	20 – 34	5	55,6
2	35 – 49	3	33,3
3	50 – 64	1	11,1
	Total	9	100,0

Tabel 2
Distribution of Respondents by Gender

No	Gender	requency	Percent (%)
1	Male	6	66,7
2	Female	3	33,3
	Total	9	100,0

2. **The amount of AFB is based on storage time**

Tabel 3
The amount of BTA

No	Immediately	3 hours delay	6 hour delay
1	2322	2315	2310
2	1562	1560	1555
3	410	405	400
4	55	52	50
5	750	747	745
6	70	67	65
7	556	553	550
8	2153	2150	2147
9	85	83	80

3. Data Normality Test

Normality test is a test conducted with the aim to assess whether the data is distributed normally or not. Normality test is useful for determining data that has been collected in normal distribution or taken from a normal population.

The normality test can use the *Kolmogorov Smirnov* test if the respondent is> 50 samples, because in this study only 9 respondents, the data normality test uses the *Shapiro-Wilk* test

Tabel 4
Uji Shapiro-wilk

	Shapiro-Wilk	
	BTA Storage Time	Sig.
Number of Bacteria	Immediately	0.061
	3 hours delay	0.060
	6 hour delay	0.060

4. Data Homogeneity Test

Homogeneity test data is used to determine whether several population variants are the same or not.

Tabel 5
Data Homogeneity Test

Homogenity of Variances Test	
Levene Statistic	Sig.
	1,000

4. Anova Test

ANOVA Test is a statistical test that aims to compare the variance of 3 groups of samples or more

Tabel 6
Analysis Test Of Varians

ANOVA

	BTA Amount				
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	206.741	2	103.370	.000	1.000
Within Groups	19399279.560	24	808303.315		
Total	19399486.300	26			

Discussion

Tuberculosis (TB) is an infectious disease that is usually caused by the bacterium *mycobacterium tuberculosis* (MTB). Tuberculosis generally attacks the lungs, but can also affect other parts of the body. Most infections have no symptoms, in this case known as latent TB. About 10% of latent infections develop into active diseases which if not treated can kill around half of them.

Pulmonary TB disease is transmitted through the air *(droplet nuclei),* when the patient coughs, sneezes or talks, the germ of pulmonary TB in the form of *droplets* will scatter in the air. *Droplets* were very small then dries quickly and contains *droplets* containing TB bacteria lungs. Pulmonary TB bacteria can stay in the air for several hours, so that sooner or later *droplets* containing the bacteria Tb Lung will be inhaled and lodged in someone's lungs, then tuberculosis will begin to divide, or multiply, from this point where infection occurs.

Bacteria *Tuberculosis* (TB) often attacks the lungs with classical symptoms such as cough, weight loss, no appetite, fever, night sweats, coughing up blood, chest pain, and weakness. This type of cough is also common with phlegm that lasts for more than 21 days.

To find out the results of the examination, it is necessary to do an AFB test, one of which is a microscopic test using sputum.

In this study, researchers used microscopic sputum examination with a 4% NaOH sample decontamination method because direct sputum examination without processing still has many disadvantages, namely, a lot of mucus and tissue that increase the sample volume, so that it will reduce the possibility of taking samples containing *mycobacterium tuberculosis*. Therefore, to improve the effectiveness of sputum microscopic examination, researchers conducted homogenization method samples with 4% NaOH so that smear will be collected in smaller volumes and will increase the likelihoodof taking samples containing germs.

Decontamination of the sample with 4% NaOH is done by diluting the sputum sample using 4% NaOH in a ratio of 1: 1 (1

ml sample: 1ml NaOH), homogenized using a turbo mixer, divortex for several seconds, ensuring that the specimen is completely mixed , then left for 15 minutes at room temperature, then added phosphate buffer saline (PBS) ph 6.8 to 7.0 to a volume of 45 ml, after that centrifuged specimens with a speed of 3000 G for 15 minutes, then the supernatant is slowly discharged and deposited 1 ml of PBS 6.8-7.0 is added. Furthermore, the decontamination results were prepared with the help of a 100 µl pipette, after the preparations were made, the preparations were then template, after which BTA Ziehl Neelsen was stained, after staining the examined microscope with 100x objective lens enlargement was carried out.

In this study, researchers used the *Analysis Of Variance (ANOVA)* test to find out whether there was an influence on the duration of sputum morning storage at room temperature on the number of acid-resistant bacteria (BTA). However, before conducting the ANOVA test , the data normality test was carried out, and the data homogeneity test was one of the requirements for the ANOVA test.

The following explanation is based on a table of research results obtained. Based on table 1 illustrated that respondents were classified into 3 age groups namely age 20-34 years as many as 5 respondents (55.6%) age 35-49 Years as many as 3 respondents (33.3%) and aged 50-64 years as much as 1 respondent (11.1%). While in the table Based on the table 2 respondents by sex depicted that respondents were male sex as many as 6 respondents (66 , 7 %) and respondents who were female as many as 3 respondents (33.3%). While in table 3 it can be seen from the table that the maximum number of bacteria in sputum that was examined immediately was 2322 BTA and the minimum number of bacteria in sputum that was examined immediately amounted to 55 AFB, while the maximum number of bacteria on sputum that was delayed by 3 hours was 2315 BTA, and the number the minimum bacteria in the sputum that was examined delayed by 3 hours was as much as 52 smear, and the maximum number of bacteria in sputum that was examined was delayed by 6 hours as much as 2310 smear and the minimum number of bacteria on sputum that was examined was delayed by 6 hours as much as 50

After obtaining the results of the inspection, the *ANOVA* statistical test is performed , but before conducting the *ANOVA* test the first thing to do is the data normality test to see whether the data is normal or not, to meet one of the *ANOVA* test requirements.

The normality test can use the *Kolmogorov Smirnov* test if the respondent is> 50 samples, because in this study only 9 respondents, the data normality test uses the *Shapiro-Wilk* test .

As based on table 4 the Shapiro Wilk test can be seen that the significance value (Sig.) Of the Immediate inspection data is 0.061, the 3-Hour Delay is 0.060 and the 6-Hour Delay is 0.060. The three variance data above shows the Sig. >0,05 . Thus the data distribution of respondents in this study were normally distributed. After the data normality test was carried out , then the data homogeneity test was carried out

Based on table 5 data homogeneity test, it is obtained the results of analysis that the significance value of the *levene test is* 1,000> 0.05, so it can be concluded that the 3 sample groups above have the same or homogeneous variance. Because the sample data of this study is normally distributed and is homogeneous, it has met the requirements of using the Parametric Statistical Test, in this case the Anova test.

After conducting ANOVA statistical test Based on the analysis result table 6, it is illustrated that the significant value of *the ANOVA Test is* 1,000> 0.05 thus it can be concluded that there is no significant difference between sputum that is examined immediately, delay 3 hours, and delay 6 hours.

From the results of research conducted at the Makassar Public Lung Health Center (BBKPMM), it was found that the duration of sputum phlegm storage at room temperature had no effect on the amount of BTA (ρ = 1,000). Thus, the alternative hypothesis in this study was rejected and the null hypothesis was accepted.

In this study, researchers compared morning phlegm that was examined immediately, delayed by 3 hours, and delayed by 6 hours to see the effect of duration of storage of morning phlegm on the number of acid-resistant bacteria (BTA).

This study is in line with previous studies, namely research conducted by Adrika in 2018 which revealed that there was no significant effect between direct sputum

samples and 24-hour delay samples on the number of acid-resistant bacteria (BTA)

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

Researchers concluded that the duration of morning phlegm storage for 6 hours, at room temperature did not affect the number of Acid Resistant Bacteria (ρ = 1,000) thus, the alternative hypothesis in this study was rejected, and the null hypothesis was accepted.

Suggestion

Based on the conclusions above, researchers suggest that it is best to continue using morning phlegm that is checked immediately. However, in certain circumstances sputum morning may be postponed for inspection up to 6 hours.

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