Comparison of N-acetyl-L-cysteine-sodium hydroxide and Modified Petroff’s Decontamination Methods for *Mycobacterium tuberculosis* Culture

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Indonesia is one of 22 countries with a high incidence of tuberculosis in the world, particularly related to TB-HIV and MDR-TB cases. Contamination of normal flora from nasopharyngeal tract is the main problem to isolate Mycobacterium tuberculosis (MTB) from sputum. Therefore, a safe solution to decontaminate sputum without killing MTB bacilli is needed. N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) and Petroff’s methods which were modified with NaOH (4%) are widely used in laboratories. The present study, we will evaluate the different of these methods. Of the 110 sputum samples were collected from suspected cases of Pulmonary TB, and the decontamination of sputum by these methods was performed before acid-fast bacillus (AFB) smear and culture in Lowensteins Jensen slant medium. The positive culture was validated by chromatography test for detecting the antigen of MPT-64 and PNB. Based on the investigation, it has been shown that neither NALC-NaOH (71%) nor modified Petroff’s methods (66%) had a significant effect on the positivity rate of AFB smear. However, the contamination on culture was significantly higher in samples treated with NALC-NaOH (21%) compared to Modified Petroff methods (13%) (p< 0.05). In addition, the proportion of positive culture in NALC-NaOH was lower than Modified Petroff. In conclusion, our study proved that Modified Petroff methods are still more effective on sputum decontamination than NALC-NaOH based on the positivity rate of MTB culture. Though, not significantly different on AFB microscopic examination.

Key words: decontamination, Modified Petroff’s, MTB, NALC-NaOH

Tuberculosis (TB) is one of the deadliest infectious disease which caused by *Mycobacterium tuberculosis*. TB is a major air-borne disease in human. It remains a major worldwide health problem with global mortality ranging from 1.6 to 2.2 million lives per year (WHO 2016). Direct smear microscopy for acid fast bacilli (AFB) is rapid, inexpensive, highly specific, and capable of identifying the most infectious cases of TB. The only disadvantage of this method is low sensitivity (varying from 50 to 80%) relative to culture (Forbes *et al.* 2010).

The Gold standard for diagnosing pulmonary TB remains in culturing. Decontamination of clinical specimens such as sputum is an important and critical step in the isolation of mycobacteria, to get better
results. However, bacterial and fungal contamination of the culture frequently may interfere with the interpretation data result. This condition can result the need to repeat the culture and lead to reduce the effectiveness of culture as a method of diagnosis of tuberculosis, because it will take more time for a colony to appear (Bruchfeld et al. 2000; Sjahurachman et al. 2012; Korean CDC 2014). Contamination of culture and its harmful effect can be prevented by decontamination of sputum sample. Decontamination and concentration of sputum samples by Modified Petroff's method is one of the most commonly used methods for M. tuberculosis culture. However, N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution may recommended as a gentle but effective digesting and decontaminating agent. Addition of a large volume of M. phosphate buffer (PB) with pH 6.8 makes strong shift in pH, washes the specimen, dilutes toxic substances and decreases the specific gravity of the specimen so that centrifugation is more effective. Therefore, it is generally accepted that the NALC-NaOH method of Kent and Kubica et al. should be given preference over the modified Petroff's methods. But, the technical and procedural factors may influence the sensitivity of each method (Peres et al. 2009). Studies in different laboratory may give different results since the result of decontamination may be influenced by various technical factors (Forbes et al. 2010; Peres et al. 2009; Sjahurachman et al. 2012). The aim of this study is to compare the NACL-NaOH and Modified Petroff method with 4% NaOH for getting better result on microscopic examination of AFB and MTB culture.

MATERIALS AND METHODS

Sputum Samples. The cross-sectional study was conducted in the tuberculosis laboratory, Microbiology Departement, Faculty of Medicine, Universitas Indonesia, from December 2016 to July 2017. Of the 110 sputum samples were isolated from suspected case of pulmonary tuberculosis. The samples then be decontaminated with NALC-NaOH or Modified Petroff's methods. Microscopic Examination. Microscopic examination was performed before and after decontamination. AFB smear was conducted using Ziehl-Neelsen technique recommended by World Health Organization (WHO 2016; Tripathi et al. 2014; Burdz et al. 2003). Specimens for digestion and decontamination were mixed well using vortex and equally divided into two parts, and each treated by NALC-NaOH and modified Petroff's method before inoculated it directly on Lowenstein-Jensen (LJ) medium (Peres et al. 2009; Tripathi et al. 2014).

NALC-NaOH Method. NALC-2% sodium hydroxide-sodium citrate solution was prepared as described by Kent and Kubica (Tripathi et al. 2014). An equal volume of NALC-NaOH citrate reagent was added into 3-5 ml of seeded sputum sample, and vortexed briefly for 30 seconds. The samples then were incubated for 15 minutes at room temperature and added by 0.067 M of phosphate buffer (pH 6.8) until the volume reach 50 ml. After homogenization, the sputum samples were pelleted by centrifugate it at 3,000g for 15-20 minutes. The supernatant was discarded, and the pellet was resuspended with 1 ml of PBS. The smear was made and a 0.5 ml of cell suspension was inoculated on LJ slopes. The culture slants were incubated at 35-37°C.

Modified Petroff's Method. In brief, 3-5 ml of sputum was homogenized for 15 minutes in a shaker using an equal volume of 4% NaOH. After centrifugation at 3,000g for 15-20 minutes, the seeds were neutralized with 20 ml of sterile distilled water or
The samples were again centrifuged at 3,000g for 15-20 minutes. From the sediment, LJ medium was inoculated and smear was made. The culture slants were incubated at 35-37°C (Pathak et al. 1973; Tripathi et al. 2014). All slopes were observed daily for first week and weekly for 8 weeks. Some parameters such as, growth rate, optimum temperature, colony morphology and pigmentation were also observed as growth parameters. The chromatography test was using to detect the antigen of MPT-64 and PNB tests. The absence of growth at the end of 8th weeks was regarded as negative culture. Contamination, if any, was recorded separately. The number of culture failures for a certain decontamination method, included the number of specimens with negative culture as well as number of contaminated cultures. The data acquired from the study is processed using SPSS software version 20. Bivariate analysis was done using Chi-square test.

**RESULT**

Based on direct microscopy, out of 110 samples, 88 (80%) were smear positive and 22 (20%) were negative. The smear was carried out again after decontamination, and the result showed that 71 samples (71%) were positive by NALC-NaOH and 73 samples (66%) were positive modified Petroff's methods (Table 1).

The total number of culture failures (which includes both contamination and negative cultures) were 33 (30%) in NALC-NaOH as against 38 (35%) in modified Petroff's methods as shown in Table 2. The contamination rate was higher NALC-NaOH 23 (21%), whereas it was lowest in modified Petroff’s methods 14 (13%) (Table 2).

A significant difference (p<0.05) in the proportion of contaminated and uncontaminated culture was shown between those treated with NALC-NaOH and Modified Petroff. Higher proportion of contaminated culture is observed from group treated with NALC-NaOH in comparison to Modified Petroff (Table 3). However, the proportions of uncontaminated culture with positive and negative results show no significant difference between group treated with NALC-NaOH and Modified Petroff (Table 4).

**DISCUSSION**

Sputum culture method is an important tool for TB control programs because it is more sensitive than smear microscopy in diagnosing TB. The culture also facilitates for drug susceptibility testing, but since sputum samples pass through the oropharynx tract during collection, culture contamination limits the diagnostic yield of sputum culture for TB (WHO 2016; Forbes et al. 2010).

Decontamination process for removing bacteria and yeast in order to isolate mycobacteria in the sputum, unfortunately may also kill mycobacteria. The percentage of killed organisms will vary according to the method used and also the population of mycobacteria in the specimen. In this study the use of NALC-NaOH for sputum digestion, as in Kent and Kubita method, will have final concentration about 2% NaOH. Such concentration will only give less destructive to mycobacteria compare to the used of 4% NaOH in Petroff’s method (GLI 2014; Tripathi et al. 2014; Burdz et al. 2003).

In the analysis of 110 samples, the decontaminated by the NALC-NaOH method provided isolation of M. tuberculosis in a lowest percentage (65%) compared modified Petroff’s method (70%). This is in accordance with the research of Sharma et al. in 2012 showed that the proportion of positive culture results was greater in samples processed with NALC-NaOH 63.7% than samples processed with Modified Petroff 46.7%. Meanwhile, from the research of Chatterjee et al. In 2013 showed that the proportion of positive culture results was greater either in samples processed with NALC-NaOH was 62.7% than samples processed with Modified Petroff 58.5% (Chaudhary et al. 2013). Meanwhile, the research results of Pathak SK et al. in 1973 showed that the proportion of positive culture results was 79.4% for NALC-NaOH. This figure is much greater than the proportion of positive culture results for NALC-NaOH which was shown well by the study of Sharma et al. 2012 and Chatterjee et al. 2013.

The probable reason are, 4% NaOH is used for modified Petroff’s method as compared NALC-NaOH method which uses 2% NaOH while concentrating the sputum may kill or seriously injure few Mycobacteria (Korean CDC 2014). Hence recovery by NALC-NaOH was faster and better than modified Petroff's method. In our study we found smear positivity higher than the culture positivity. The reason might be that microscopy sometimes gives false positive results and in our condition, it cannot distinguish between dead and live bacteria. In such cases, the patients might be treated with antitubercular drugs and in the microscopy of these samples, the AFB might be dead. For these reason, the dead isolates did not grow in the L-J culture.
This also reveals that AFB microscopy does not always give accurate results for the diagnosis of TB (Sharma et al. 2012; GLI 2014). The contamination rate by NALC-NaOH method was 21% in our study which is higher than that reported by other workers. Sharma et al. 2012 reported 13.2% while Chatterjee et al. in 2013 reported 4.98%. Several studies show the contamination rate by modified Petroff’s was 13% in our study which is lower than that reported by other workers. Sharma et al. 2012 reported 23.1% while Chaudary et al. in 2013 reported 8% and Tripathi et al. in 2014 12%. In our study the NALC-NaOH method for AFB smear and culture improves the sensitivity when compared with modified Petroff’s method. In our study Modified Petroff method has lower contamination rate than NALC-NaOH, either for culture using LJ medium NALC-NaOH method is not suitable for routine use. The modified Petroff’s method for AFB smear and culture improves the sensitivity when compared with the NALC-NaOH method.

In conclusion, the Modified Petroff methods is more effective than NALC-NaOH to prevent contamination from highly contaminated sputum. However, the positivity result of microscopic examination of AFB was not significantly different between this two methods.

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