

Modified Petroff's Method: an Excellent Simplified Decontamination Technique in Comparison with Petroff's Method

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Research Article

Abstract: Tuberculosis is a major public health problem. In India, about 40% of the population is infected with TB bacillus. For diagnosis of TB, smear microscopy is still the most used due to its simplicity though it lacks sensitivity as compared with culture. This study compared the original Petroff's method with Modified Petroff's method. Spot and early morning sputum samples of 225 suspected tuberculosis cases were collected and processed by petroff's and Modified Petroff's method separately. Treated samples were cultured in Lowenstein-Jensen media. Out of 225 processed sputum specimens, 123 (54.67%) were smear positive by direct, 127 (56.44%) by Petroff's method and 129 (57.33%) by Modified Petroff's method. The number of culture positives by Modified Petroff's method was 55.56%, by Petroff's method were 53.33% and 45.33% by Direct method. Culture positives by Modified Petroff's method compared with Direct method are statistically significant ($P=0.0249$, Chi square test). Negative culture and contamination rate was minimum with Modified Petroff's method. Modified Petroff's method requires lesser time, has lower contamination rate and higher yield in culture positivity as compared with Petroff's method, making it a more suitable and better method of concentration and decontamination.

Keyword: Tuberculosis, decontamination, Petroff's method, Modified Petroff's method.

Introduction

Tuberculosis is one of the major health problems particularly in developing countries. Mycobacterium tuberculosis is the most likely etiologic agent of chronic lower respiratory tract infection. M. tuberculosis infection of the lung is so common worldwide that it must be considered in every instance of community acquired pneumonia [1]. Mycobacterium tuberculosis infects almost the third part of the world population killing around two million people worldwide each year. About 80% of the global TB burden is in low-income countries, where pulmonary disease and transmission are serious public health problems [2, 3]. As per the WHO Global TB Report 2013(4), there were an estimated 8.6 million

incident cases of TB globally in 2012 with 1.3 million deaths. Out of this estimated global annual incidence of TB cases, 2 million were estimated to have occurred in India, thus contributing to a fifth of the global burden of TB. It is estimated that about 40% of Indian population is infected with TB bacillus [5]. For definitive diagnosis of pulmonary tuberculosis identification of mycobacteria in sputum by microscopy and culture is essential [6]. The isolation of mycobacteria from specimens contaminated with normal flora like sputum poses a problem and these specimens have to be treated to kill various non-acid fast organisms by decontamination techniques. A great variety of decontamination methods are in existence and different laboratories use different decontamination techniques but no single method is entirely satisfactory [7]. Currently, for diagnosis of TB, smear microscopy is still the most used amongst all methods employed worldwide due to its simplicity, low cost, speed and minimal requirement of equipment and technical skills. Though it lacks sensitivity since a load of about 5,000 to 10,000 bacilli/ml of specimen is required to give a positive result after Ziehl – Neelsen staining [8]. In developing countries, culture on Lowenstein-Jensen solid medium is the gold standard for microbiological diagnosis of TB and requires about 10 bacilli/ml of specimen for recovery of mycobacteria. The slow growth rate of the pathogen leads to a delay of 4-6 weeks in obtaining a definitive diagnosis [8, 9]. Since sputum microscopy is the cornerstone of TB diagnosis, a more sensitive smear microscopy and decontamination method would both be useful in clinical laboratories so as to achieve increased, accurate and rapid TB diagnosis [3]. In the present study, Petroff's method is compared with Modified Petroff's method.

Materials and Methods

This study was carried out at the Microbiology Department of Medical College and Research centre, Bhopal, Central India from October 2012 to September 2013 after getting the approval from the Institutional Ethical Committee. 225 sputum samples of suspected pulmonary tuberculosis cases were selected for the study. Spot and early morning samples of sputum were collected in 2 sterile wide mouth containers and were processed and graded on the same day as per Revised National Tuberculosis Control Programme (RNTCP) guidelines [10]. Only sputum samples with a volume of 5 ml or more were included in the study. Samples were labeled as saliva, mucoid, mucopurulent, purulent or blood stained according to their physical appearance. They were vortex mixed and processed in a biosafety cabinet level II for acid-fast staining and decontamination by both Petroff's method and Modified Petroff's method. Sputum was divided into two equal parts, one part was used for Petroff's method and other part was used for Modified Petroff's method. All the samples were also inoculated directly on Lowenstein Jensen (LJ) medium without doing decontamination method and after doing decontamination by both methods.

Petroff's Method: The sputum was transferred to a sterile test tube and equal amount of sterile 4% NaOH was added to it. The tube was incubated at 37°C for 30 minutes with vigorous shaking every 5 minutes. The mixture was centrifuged at 3,000 rpm for 30 minutes and supernatant poured off. The deposit was neutralized by N/10HCL using a drop of phenol red as indicator. L.J. slope was inoculated and incubated at 37°C [11].

Modified Petroff's Method: 3-5 ml of sputum was homogenized for 15 min in a shaker using an equal volume of 4% NaOH. After centrifugation at 3,000 rpm for 15 min, the deposit was neutralized with 20 ml of

sterile distilled water. The samples were again centrifuged at 3000 rpm for 15 mins. From the sediment, LJ medium was inoculated and smear was made. The culture slants were incubated at 37°C [2,12].

All Slopes were observed for occurrence of growth daily for first week and then at weekly intervals for 8 weeks. The isolates were identified by following tests such as rate of growth, pigment production, colony characteristics, niacin test, nitrate reduction test which confirmed that all isolates are *M. tuberculosis*.

Absence of growth at the end of 8th week 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 along with the number of contaminated cultures.

Statistical analysis

P value was reported and a value of $P < 0.05$ was considered as significant. The statistical analysis was performed using Chi square test.

Results

Out of 225 processed sputum specimens, 123 (54.67%) were smear positive by direct, 127 (56.44%) were smear positive by Petroff's and 129 (57.33%) were smear positive by modified Petroff's method. 125 (55.56%) positive culture strains were obtained by Modified Petroff's method. 102 (45.33%) and 120 (53.33%) strains were culture positive by direct and Petroff's method [Table]. Table 2 shows weekwise growth on LJ medium. Table 3 shows total number of culture failure which included both negative culture and contaminated slopes. Negative culture and contaminated slopes. Negative culture and contamination rate was minimum with Modified Petroff's method [Table 3].

Table 1: Distribution of direct smear examination and culture results

Result	Microscopy*			Culture		
	Direct (%)	Pettoff's (%)	Modified Petroff's (%)	Direct [#] (%)	Pettoff's (%)	Modified Petroff's [#] (%)
Positive	123 (54.67)	127 (56.44)	129 (57.33)	102 (45.33)	120 (53.33)	125 (55.56)
Negative	102 (45.33)	98 (43.56)	96 (42.67)	77 (34.22)	74 (32.89)	73 (32.44)
Contamination	-	-	-	46(20.45)	31 (13.78)	27 (12)
Total	225	225	225	225	225	225

(Chi square test * $P=0.8450$, [#] $P=0.0249$)

Table 2: Weekwise growth in the 2 decontamination methods

Method	Total Samples	1 st week (%)	2 nd week (%)	3 rd week (%)	4 th week (%)	5 th week (%)	6 th week (%)	7 th week (%)	8 th week (%)
Direct	225	0	4 (1.77)	56 (24.88)	69 (30.66)	78 (34.66)	96 (42.66)	98 (43.55)	102 (45.33)
Petroff's method	225	0	9 (4)	58 (25.77)	74 (32.88)	96 (42.66)	115 (51.11)	118 (52.44)	120 (53.33)
Modified Petroff's method	225	0	19 (8.44)	73 (32.44)	89 (39.55)	112 (49.77)	120 (53.33)	123 (54.66)	125 (55.56)

Table 3: Comparison of two decontamination methods for rate of contamination, negative culture and culture positives

Concentration method	No. of contaminated slopes	Negative cultures (No of slopes with no growth upto 8 weeks)	Total culture failures (%)
Direct	46 (20.45)	77(34.22)	123 (54.67)
Petroff's	31 (13.78)	74(32.89)	105 (46.67)
Modified Petroff's	27(12)	73 (32.44)	100(44.44)

Discussion

For diagnosing Tuberculosis, smear microscopy and sputum culture are important tools. Sputum culture being the gold standard is a more sensitive method compared with microscopy as it detects as few as 10–100 bacilli/ml. It also facilitates drug susceptibility testing. But contamination of culture specimens limits the diagnostic yield of sputum culture [13]. Sputum decontamination methods are used for isolation of mycobacteria and to kill the oral bacteria present in the specimen. These decontamination methods also kill the mycobacteria and the percentage of organisms killed varies according to the method used and the mycobacterial species present in the specimen [14]. Several studies have reported that the use of decontamination methods with increased concentration of NaOH (3–4%) reduced bacterial contamination rates, but its toxicity to mycobacteria may sometimes yield negative culture results [15]. In the Present study, we used Modified Petroff's and Petroff's method of sputum digestion. In the original Petroff's method sputum is digested with NaOH and the centrifuged deposit is neutralized with HCl before culture. In Modified Petroff's method digestion is arrested by dilution with water and centrifuged deposit is used for culture which is simpler, safer and seems to give a greater number of positive smears and culture for mycobacteria with minimum overgrowth by contaminants. Contamination rate was also reduced when Modified Petroff's method was used for digestion and decontamination. Ghosh et al [16] also reported similar findings. Modified Petroff's method is used widely in developing countries because of its relative simplicity and the fact that only one reagent is required and is easy to obtain. Moreover, the time of exposure to 4% NaOH is reduced and not only that NaOH is removed by washing with distilled water. Whereas in original Petroff's method, the time of exposure to 4% NaOH is more and NaOH is neutralized by adding 8% HCl and phenol red indicator and if neutralization is not carefully carried out the medium may be acidic which is also deleterious to the mycobacteria. In the present study, maximum number of positive cultures (55.56%) was obtained by Modified Petroff's method compared with Petroff's method (53.33%). Chaudhari et al [6] reported a

higher culture positivity of 70% by Modified Petroff's method. Stewart et al [17] also reported 64% culture positivity. In our study we found smear positivity higher than the culture positivity [Table 1]. The probable reason might be that microscopy may sometimes give false positivity and cannot distinguish between dead and live bacilli. The patient might be treated with antitubercular drugs and the microscopy of these patients might reveal the dead bacilli. For these reasons, the dead isolates did not grow in LJ culture medium. Thus for the diagnosis of TB, AFB microscopy alone should not be used as it does not always give accurate results and in doubtful cases LJ culture should be used for confirmation. The number of culture positives by Modified Petroff's method was 55.56%, by Petroff's method were 53.33% and 45.33% by Direct method. Culture positives by Modified Petroff's method compared with Direct method are statistically significant ($P=0.249$, Chi square test). Although culture positivity by Petroff's method compared with Direct method ($P=0.0769$, Chi square test) and sputum for AFB Positivity by Concentration methods (Modified Petroff's and Petroff's method) is statistically insignificant ($P=0.8450$, Chi square test), but under programme conditions (RNTCP) it has enormous value as these sputum positive TB cases must be treated by Category I DOTS because sputum positive cases can transmit infection in the community [Table 1]. Hence Modified Petroff's method is a better option compared with Petroff's method. In this study we observed that a higher growth was obtained with modified Petroff's method during 5th week (49.77%), 6th week (53.33%), 7th (54.66%) and 8th week (55.55%) as compare with Petroff's method (Table 2). Six cases were found to be smear positive by Modified Petroff's method and 4 cases by Petroff's method as compared with Direct method in smear microscopy. False results in AFB sputum smear microscopy during 'diagnosis' and 'follow up' of treatment affect the quality of laboratory services offered to the patients. False negative results lead to a patient being denied TB treatment and subsequent risk of spreading the TB disease in the society. False negative results also lead to incomplete treatment and being wrongly declared as cured (18). The contamination rate by Modified Petroff's method was 12% (Table 3).

Chaudhari et al (6) reported contamination rate of 8% by Modified Petroff's method. Somoskovi et al (19) have reported contamination rates ranging from 1.5 to 13.3%.¹⁹ Our findings are comparable with Somoskovi et al.

Conclusion

To conclude, Modified Petroff's method requires lesser time, lower contamination rate and higher yield in culture positivity as compared with Petroff's method, making it a more suitable and better method of concentration and decontamination. This study reiterates the importance of specimen concentration and decontamination, without which the contamination rates may reach unacceptable rates leading to culture loss and poor isolation rates. Sputum microscopy likely the only diagnostic test that can be widely implemented in short term to improve tuberculosis case finding. A single sputum positive case missed by Direct microscopy can infect 10-15 persons/year and thus transmit infection in the community resulting in patient suffering and death. Hence Concentration method such as modified Petroff's method with higher smear and culture positivity, lower contamination rate and less deleterious effect on mycobacterium should be recommended in the RNTCP in order to reduce the rate of transmission of TB in the community. To assess the exact efficiency of culture positivity, growth rate, grading of growth on LJ and rate of contamination by Modified Petroff's method more number of samples should be analyzed.

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