ORIGINAL ARTICLE

Evaluation of modified petroff's method and manual MGIT culture system to facilitate detection of acid-fast bacilli in smear-negative presumptive cases of pulmonary tuberculosis

Sana Jamali[1], Richa[2], Vinod K. Srivastava[2]

[1]Associate Professor, Department of Microbiology, Integral Institute of Medical Sciences & Research, Lucknow, Uttar Pradesh, [2]PhD Scholar (Microbiology), Dr SN Medical College, Jodhpur, Rajasthan, [3]Associate Professor, Department of TB & Chest Diseases, Integral Institute of Medical Sciences Research, Lucknow, Uttar Pradesh.

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Corresponding Author

Dr. V. K. Srivastava, Associate Professor of TB & Chest Diseases, Integral Institute of Medical Sciences & Research, Lucknow - 226026 Uttar Pradesh
E Mail ID: drvksrivastavachest@gmail.com

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Abstract

Background: Tuberculosis is a major public health problem in India. According to RNTCP guidelines, all efforts are to be made to diagnose tuberculosis on the basis of detection of AFB/ MTB in the clinical specimen; hence all those cases where AFB/ MTB have not been detected are presumptive cases of tuberculosis. In this background, the present study aims to detect AFB/ MTB in the sputum of clinico-radiologically presumptive cases of pulmonary tuberculosis using direct ZN microscopy, Modified Petroff's method followed by ZN microscopy & MGIT liquid culture method. **Materials and Methods:** A total of 94 sputum samples which were negative for AFB by direct ZN microscopy at the DMC of the institute, were subjected to Modified Petroff's method followed by microscopy & MGIT liquid culture test to find out any additional yield of bacteriologically confirmed disease. **Result**: Out of 94 specimens, 20 (21.28%) were positive for AFB on ZN microscopy post Modified Petroff's method. Among all the specimens, 30 (31.91%) were found to be positive by MGIT liquid culture method with average time to detection growth (TTD) around 16.73 days (2-38 days). **Conclusion**: Modified Petroff's method followed by ZN microscopy & MGIT test for mycobacterial culture can improve case detection.

Keywords

Modified Petroff; S method; Concentrated smear technique; MGIT; Tuberculosis

Introduction

Tuberculosis is basically a bacterial diagnosis. Many diseases give similar clinical and radiological picture. Even most experienced clinician is liable to make mistake in diagnosing tuberculosis on clinical & radiological basis, resulting in over/under diagnosis. Hence demonstration of offending organism i.e. AFB/Mycobacterium tuberculosis in the clinical specimen is of utmost importance to put a label of tuberculosis as a diagnosis.

Various diagnostic techniques like microscopy, culture, PCR based tests are available to demonstrate presence of AFB/ Mycobacterium tuberculosis in the clinical specimen. Today, even in

the programmed conditions such as RNTCP, all these diagnostic modalities are being used. Although direct microscopy is less sensitive (when the bacterial load is less in the specimen) but its importance could not be undermined, because it is the cheapest, very easy & quick method of diagnosing tuberculosis. It can be performed in a remote place by a trained microscopist. To enhance the outcome of microscopy, various methods have been tried i.e. Modified Petroff's method followed by ZN microscopy.

The MGIT (Mycobacterial Growth Indicator Tube) test is a non radioactive detection system which uses fluorochrome based method for detection of Mycobacteria in the clinical specimen (except blood & urine). This system is useful in early detection of Mycobacterial growth with an average time to detection of 7-12 days.

Aims & Objectives

1. To evaluate concentrated smear technique and micro MGIT system for detection of acid-fast bacilli among smear-negative presumptive cases of pulmonary tuberculosis.
2. To identify the additional yield of active pulmonary tuberculosis cases through ZN microscopy after concentration of the specimen by Modified Petroff's Method and MGIT liquid culture system.

Material & Methods

A total of 94 patients participated in the study. Sputum samples were collected from patients, who were clinico-radiologically suspected new cases of Pulmonary Tuberculosis or old treated cases of Pulmonary Tuberculosis and whose two sputum samples (spot & morning) were found negative for AFB by conventional ZN microscopy in the DMC functioning in the institute under RNTCP.

Modified Petroffs method: All the samples were treated with N-Acetyl-L-Cystein (NALC), 4% Sodium Hydroxide (NaOH) and 2.9% Sodium Citrate solution. If few clumps were noticed an extra pinch of NaOH crystal were mixed in the liquefied sputum. After 15 minutes the treated sputum was centrifuged at 3600g for 15 minutes. Supernatant was poured out and sediment was re-suspended in to 1-2 ml phosphate buffer and slides were prepared for ZN Microscopy.

Culture by manual MGIT system (BBL MGIT): MGI Tubes manufactured by Becton Dickinson were used in the study. Tubes contained 7ml modified middle brook 7H9 broth. Before inoculating the tubes with 0.5 ml of decontaminated and concentrated specimen by Modified Petroff's method, an addition of OADC enrichment and PANTA (Polymyxin-B, Amphotericin-B, Nalidixic acid, Trimethoprim, Azlocillin) was made in the MGIT tube. The inoculated MGIT tubes were kept in the incubator at 37 degree Centigrade for a maximum period of 6 weeks. Tubes were checked daily for presence of turbidity, granularity and were read on BACTEC manual MGIT fluorescence reader. Tubes showing reading above 14, up to 20 marks were considered positive & those below 13 were considered negative. From all the positive MGIT tubes smears were prepared and ZN staining was done followed by microscopy to see the presence of acid-fast bacilli. A portion of the broth was also inoculated on blood agar to check for contamination. For species identification SD BIOLINE TB Ag MPT64 kit was used for rapid immune-chromatographic identification of M. tuberculosis complex.

Results

In the present study, after subjecting total 94 specimens to modified Petroff's method followed by ZN microscopy, 20 (21.3%) were found to be positive for acid-fast bacilli. 10.63% (10/94) additional cases who were negative for AFB on microscopy after Modified Petroff's method were detected by MGIT liquid culture system (Table 1). Hence, out of 94 cases, 30 (32%) patients were found to be positive for Mycobacterium tuberculosis complex by micro MGIT liquid culture test with average Time to Detection growth (TTD) around 16.7 days (2-38 days). Sensitivity and specificity of Microscopy post Modified Petroff's method with MGIT liquid culture method as gold standard was calculated to be 33.3% and 84.4% respectively (Table 2). Smear positivity as per RNTCP criteria was scanty in 45% of the specimens followed by 1+ (40%), 2+ (10%), and 3+ (5%).

Out of 94 samples, 63 (67%) were from males while 31 (33%) were from females. Out of them respectively 16 (25.4%) and 14 (45.2%) were MGIT positive for MTB which is statistically significant with respect to gender (p=0.0546).

The micro MGIT culture positivity was maximum in the age group of 15-44 years (35.4%) followed by 45 and above age group (30.6%). MGIT positive patients from rural area were 33% while it was 22.2% in urban area patients. Below poverty line (BPL) patients had

positive MGIT in 32.9% while it was 29.2% in above poverty line (APL) group. Unmarried patients showed positive result in 50% while it was 29.8% for married one. However statistically no significant association has been found with age group, area of residence, SE status and marital status of the patients and MGIT positivity. Association of MGIT positive status and its relationship with education, employment and various mal habits have been studied and results are shown in the following table 3. More number of untreated cases were found MGIT positive (53.6%) than previously treated patients (8%). Association between micro MGIT culture positivity and past history of TB was found to be statistically significant (Table 3).

Discussion

Tuberculosis is the first infectious disease declared as a global health emergency by the World Health Organization (1). Early diagnosis and prompt treatment is necessary to limit the spread of infection. Although most of the sputum positive cases are diagnosed by observing acid fast bacilli in ZN stained smear and culture in LJ media. The present study was under taken to find out the role of Modified Petroffs method followed by ZN microscopy and BBL Micro MGIT liquid culture method for bacteriological bacteriological confirmation of pulmonary tuberculosis among direct sputum smear-negative (Z.N method) patients.

Sputum negative pulmonary tuberculosis constitutes about 50% of all new cases of pulmonary tuberculosis. Although the relative transmission rate of smear negative tuberculosis is lower than that of smear positive cases, it is still responsible for 17% of tuberculosis transmission (2). If bacillary load in the clinical specimen is low, conventional laboratory techniques like direct microscopy are less sensitive (3) and going for culture on a solid media is a time-consuming process for the diagnosis of tuberculosis. Therefore, it is the need of time to develop new techniques for rapid identification of the Mycobacterium tuberculosis. Recently, attention has been devoted to latest liquid culture diagnostic processes due to their high sensitivity, specificity, rapidity and accuracy. Culture remains the gold standard for diagnosis of mycobacterial infections, although it is time consuming and prone to contamination (4, 5).

Several manual and automated systems have been introduced specifically to reduce the time of detec¬tion and identification of mycobacteria in clinical speci¬mens. The Mycobacteria Growth Indicator Tube (MGIT) (Becton Dickinson) provides rapid recovery of mycobac¬teria from clinical specimens (6).

In the present study out of total 94 cases, 20 AFB positive cases (21.3%) were detected by modified Petroffs method ZN microscopy and 30 including 20 detected by ZN microscopy (31.91%) patients were found to be positive by MGIT liquid culture method (statistically significant, p=<0.0001).

The average time to detection growth (TTD) was found to be 16.73 days (2-38 days). Similar findings were also reported by other investigators. Mistry et al., in the year 2016 reported that the average TTD by (Manual/Micro) MGIT was 13 - 20 days (7). Rishi et al. 2007 found the positivity rate of 34.10% (88/258) on M 960 system and average time to detect growth (TTD) was 9.66 days (8). Rawat et al., and Rodrigues et al.,reported slightly higher positivity by MGIT 960 system ie 51.9% and 41% respectively, but these are from the total samples (smear positive & negative both) (9, 10). The TTD in smear negative specimen was reported as 16 days by Rodrigues et al., (11). In another study for AFB smear-negative specimens, slightly lower rate of positivity (19.8%) by MGIT was found whereas the corresponding time of detection (TTD) was 13.2 days with the BBL MGIT system and 35.3 days with LJ culture (12).

Mistry et al., from Surat reported that negative samples required average of 20 days for detection of mycobacteria which was statistically significant ($p < 0.001$) (7).

In the present study, a higher number of male 67.02% (63/94) were positive for mycobacteria as compared to females 32.98%(31/94). Similar findings were also reported by other investigators Uddin et al., from Bangladesh and Zaman et al., from Assam (13, 1).

In the present study, out of total 94 samples we detected 30 (31.91%) positive samples by MGIT out of which 20 (21.28%) were positive for AFB by Modified Petroffs ZN microscopy & MGIT also (True positive) & rest 10 (10.63%) didn't show the presence of AFB by Modified Petroff's ZN microscopy. Thus 10.63% (10/94) additional positive cases were detected by MGIT.

In our study we found the majority of the cases were within the age group 15-44years (35.48%). Almost similar finding has been reported by Chakrabartty et al., from West Bengal (14) and much higher figures 75.86% and 75% have been reported by Uddin et al., and Mashrek et al., respectively (13, 15).

Conclusion

In conclusion this study demonstrates the vital role of manual MGIT system in facilitating early recovery of mycobacteria among direct smear-negative suspected cases of tuberculosis. As shown in our study, 20 (21.28%) direct smear-negative specimens were found positive for AFB on ZN microscopy after Modified petroff's method and all these samples were found positive for mycobacteria on the Manual MGIT also (True Positive). Thus, we could add significant number of bacteriologically confirmed cases of Pulmonary Tuberculosis by modified Petroff's method followed by ZN smear microscopy especially in resource-limited settings.

Recommendation

It is recommended that in field conditions, all sputum smear negative samples may be subjected to Modified Petroff's method followed by ZN microscopy. This can be done by already working Lab Technician with same infrastructure & equipment. This can easily be replicated in the field conditions yielding high returns and saving cost of automated MGIT method or other methods to get additional confirmed cases of Pulmonary Tuberculosis. As compared to fully automated BACTEC MGIT system, manual micro MGIT system is financially more affordable for detection of mycobacteria in resource-limited health care settings with a small budget.

Limitation of the study

The major limitation of the present study is that it is a hospital based study. Other limitations are smaller sample size and short study period. Further community based studies need to be carried out so as to generalize the findings to a larger population.

Relevance of the study

Under RNTCP, sputum smear examination by ZN method is backbone of diagnosing pulmonary tuberculosis cases because this is quick, cheap & easy to perform. It requires less expertise. Sputum smear microscopy by ZN method is having sensitivity of about 60% only hence many presumptive cases of tuberculosis are not diagnosed on bacteriological basis by sputum smear microscopy. Culture for MTB on LJ takes very long time & requires special lab & expertise hence not possible in field condition. Manual MGIT liquid culture method has promising utility for rapid detection of mycobacteria, being less expensive, no costly equipment required & can be managed by short trained person. Also specimen concentration technique resulted in additional yield of positive cases. Hence concentrated specimen microscopy and Manual MGIT method can be implemented in a resource limited setting for bacteriological confirmation of presumptive cases of tuberculosis.

Authors Contribution

All authors have contributed equally in this research.

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Tables

TABLE 1 ADDITIONAL YIELD BY MODIFIED PETROFF'S ZN MICROSCOPY AND MGIT

Method	No. Positive	Percentage
Modified Petroff's Method ZN Microscopy	20	21.3
MGIT	30	31.9

TABLE 2 SENSITIVITY AND SPECIFICITY OF TESTS

Result Test		MGIT		Total
		Positive	Negative	
Modified Petroff ZN Microscopy	Positive	10	10	20
	Negative	20	54	74
	Total	30	64	94
Sensitivity of Petroff's Test = 10/30x100 = 33.3% & Specificity of Petroff's Test = 54/64x100 = 84.4%				

TABLE 3 SOCIO-DEMOGRAPHIC PROFILE OF SMEAR-NEGATIVE PRESUMPTIVE CASES OF PULMONARY TUBERCULOSIS FOUND POSITIVE BY MGIT LIQUID CULTURE METHOD

Characteristics		Number	Positive	Percentage	P value
Age	15-44	32	11	34.4	0.71
	45+	62	19	30.6	
Sex	Male	63	16	25.4	0.55
	Female	31	14	45.2	
Residence	Rural	85	28	33.0	0.51
	Urban	09	02	22.2	
SE Status	BPL	70	23	32.9	0.74
	APL	24	07	29.2	
Marital Status	Married	84	25	29.8	0.19
	Unmarried	10	05	50.0	
History of TB	Yes	53	08	15.0	0.015
	No	41	22	53.6	
Education	Illiterate	58	16	27.6	0.14
	Primary	14	04	28.6	
	Secondary	22	10	45.4	
Employment	Employed	43	07	16.3	0.003
	Unemployed	51	23	45.1	
Tobacco Use	Smoking	29	07	24.1	0.42
	Chewing	10	02	20.0	
	None	51	20	39.2	