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Utility of MGIT-960 for detection of *Mycobacterium tuberculosis* in extrapulmonary specimens.

KEYWORDS: *Mycobacterium tuberculosis*, MGIT-960, Extra-pulmonary specimens

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ABSTRACT

Background and Objective: *Extra Pulmonary Tuberculosis (EP-TB) contributes 15 to 20% cases of M. tuberculosis (MTB) in India and due to paucibacillary nature, TB often remains undiagnosed. The aim of present study was rapid diagnosis of MTB in EP samples by using Mycobacteria Growth Indicator Tube (MGIT) 960.*

Methods and Material: *A total of 101 EP specimens were processed for Acid-Fast Bacilli (AFB) smear microscopy and culture by MGIT 960. MTB and Non Tuberculous Mycobacteria (NTM) were further differentiated by SD Bioline TB Ag MPT 64 rapid assay on MGIT positive specimens.*

Results: *Out of 101 specimens, 2 (1.98%) were found smear positive, while 18 (17.82%) were positive on MGIT-960 culture. Amongst 18 positive isolates, all isolates were MTB (100%). Smear positive samples were detected on an average in 14.1 days, while smear negative samples were detected in average 18.66 days.*

Interpretation and conclusions: *Mycobacteria tuberculosis was detected in 17.82% of the total EP cases in an average of 18 days. None of the sample was positive for NTM. MGIT 960 is a good system for detection of MTB with low turn around time. The growth obtained can be further used for Drug Susceptibility Testing (DST) also.*

Introduction:
Tuberculosis caused by *Mycobacterium tuberculosis,* is a leading health problem worldwide and remains one of the major causes of death from infectious disease. Presently one third of the world population is currently infected with the TB bacillus and there were an estimated 9.0 million incident cases of TB (range, 8.6 million–9.4 million) globally, equivalent to 126 cases per 100 000 population [1]. The burden of TB is highest in Asia and Africa. About 58% of cases are in the South-East Asia and Western Pacific regions.

In India, 2.2 million tuberculosis cases occur annually, 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. The incidence of new smear positive cases is 51 per 100,000 populations. The prevalence of TB was estimated to be 176 per 100,000 populations, and the mortality rate due to TB was 22 per 100,000 populations [2].

Apart from lungs *Mycobacterium tuberculosis* can also involve other body parts also like pleura, lymph nodes, abdomen, genitourinary tract, skin, joints, bones, meninges, brain, etc. [3,4] In India EP-TB has been reported in approximately 15–20% of TB patients. [5]

EP-TB is routinely diagnosed on the basis of clinical suspicion, radiological findings, culture, histology or Polymerase Chain Reaction (PCR) based tests but due to their paucibacillary nature this disease often remains undiagnosed and, even worse, untreated. Many times extrapulmonary tuberculosis is often initially misdiagnosed as cancer. Moreover invasive procedures are required for obtaining material for investigation in extrapulmonary cases and are therefore not easily repeatable. The diagnosis of TB and other mycobacterial infections from human clinical material in the early stage with rapidity and accuracy is very importance to decrease the incidence. Microscopy and culture are generally considered to be the important methods for the laboratory diagnosis of TB. However, smear microscopy has low sensitivity in paucibacillary specimens [6]. Moreover, solid culture i.e. Löwenstein-Jensen culture (LJ) remains the gold standard for diagnosis of mycobacterial infections, although it is time consuming and contamination rate is high [7,8].

However automated liquid culture systems like MGIT 960 [9] and PCR [10] have been reported to have better sensitivity for growth and detection of mycobacteria in EP samples respectively. MGIT-960 is a fully automated system for testing of MTB via the measurement of fluorescence by a photodetector. The amount of fluorescence is inversely proportional to the oxygen level in the culture medium, indicating the consumption of oxygen due to the growth of inoculated organisms in the vials.

The present study aims to detect *Mycobacterium tuberculosis* in extrapulmonary specimens by MGIT-960.

Materials and methods:
A total of 101 extra pulmonary specimens (pleural fluid, lymph node biopsy, cerebrospinal fluid, skin biopsy, urine, stool specimens) from patients suspected of TB were received in Mycobacteriology laboratory of SMS Medical

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College, Jaipur, India during the period, January 2014 – December 2014.

Sample Processing

All non-sterile and turbid specimens (pus, lymph node, endometrium biopsy, pleural fluid, skin biopsy and urine) were processed by the standard *N*-acetyl-L-cysteine and sodium hydroxide (NALC/NaOH) method as per RNTCP guideline. While sterile body fluid samples cerebrospinal fluid (CSF), were not processed but directly inoculated into MGIT 960 after centrifugation and one drop was used for AFB smear microscopy [11,12]. Five hundred µl of processed specimens were inoculated into MGIT 960 tube as per the manufacturer's protocol and rest of the deposits were stored at -20°C [13]. Culture positive tubes were further confirmed by smear microscopy and tested by SD BIOLINE TB Ag MPT 64 Rapid (MPT 64) test to distinguish *M. tuberculosis* from NTM.

MGIT-960

The MGIT 960 culture tubes contain 7 ml of Middlebrook 7H9 broth base, to which an enrichment supplement was added according to the instructions of the manufacturer, as well as a mixture of antibiotics consisting of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (MGIT PANTA). After inoculation of each tube with 0.5 ml of the processed specimen, the tubes were entered into the MGIT 960 instrument. The vials were incubated at 37°C and were monitored automatically every 60 min for increase in fluorescence for a maximum of 6 weeks. Any sample, which was identified as positive, was removed from the instrument. From the positive tube, a smear was prepared for examination of AFB.

SD BIOLINE TB Ag MPT 64:

MPT64, an immunochromatographic test was used for discrimination of mycobacteria into MTB and NTM, as per the manufacturer's instructions [14-17]. Briefly 100 µl of the MGIT growth suspension was added in the sample well of cassette and incubated for 15 minutes at room temperature (RT). The presence of only control band (pink color) in the absence of test band was considered as negative for MPT64 antigen (NTM). Presence of both control and test band indicated a positive result and interpreted as presence of MPT64 antigen (MTB).

Results:

Amongst the 101 EP specimens, maximum number of samples belonged to patients in the age group of 30 to 40 years (n = 54), median age of the patients was 32.90 years. Maximum number of specimens were pleural fluid 28 (27.72%) followed by pus 20 (19.80%), endometrium biopsy 20 (19.80%) and CSF 15 (14.85%). (Table-1; Figure-1)

Two samples (1.98%) (One pus and one plural fluid specimen) were found to be AFB positive. Amongst all specimens, 18 (17.82%) were culture positive, 82 (81.19%) were negative and 1 (0.99%) got contaminated on MGIT 960 culture. (Table: 1).

Table 1: Diaganosis of extra pulmonary tuberculosis specimens by ZN staning and MGIT-960

S. No.	Sample type	Number of sample	Male	Female	Mean Age	Smear	MGIT Positive	Percent Positivity
1	Pus	20	10	10	32	1(1+)	5	25%
2	Lymph node	8	3	5	26.6	Negative	1	12.5%
3	CSF	15	10	5	33.7	Negative	1	6.67%
4	Endometrium biopsy	20	0	20	31	Negative	2	10%
5	Pleural fluid	28	22	6	34.6	1(1+)	7	25%
6	Skin biopsy	5	3	2	39.25	Negative	1	20%
7	Urine	5	4	1	33.2	Negative	1	20%
	Total	101	52	49		Positive = 2	18	

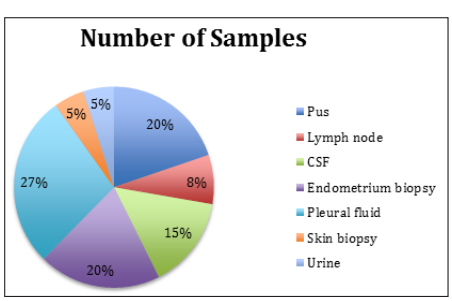

Figure 1: Figure showing total number of extrapulmonary TB suspected patients samples.

The mean turnaround time for detection (TTD) of mycobacteria in smear positive samples was 14.1 days (1+ - 11.6 days, scanty – 16.66 days) and smear negative samples was 18.66 days (16-30 days). MGIT positive specimens included 7 (38.89%) pleural fluid, 5 (27.78%) pus, 2 (11.11%) endometrium biopsy, 1 (5.56%) lymph node, 1 (5.56%) CSF, 1 (5.56%) urine and 1 (5.56%) skin biopsy sample. Maximum sample positivity was observed in pus (25%) and pleural fluid (25%) samples followed by skin biopsy (20%), urine (20%), lymph node specimens (12.5%), endometrium biopsy (10%) and CSF (6.67%) (Table-1; Figure-2). Amongst 5 pus samples which were found MGIT-960 positive, one sample was smear positive, while amongst 7 pleural fluid positive samples, one was smear positive. MPT 64 assay was carried out on all 18 MGIT positive isolates and all isolates were identified as *M tuberculosis* (MPT 64 antigen positive).

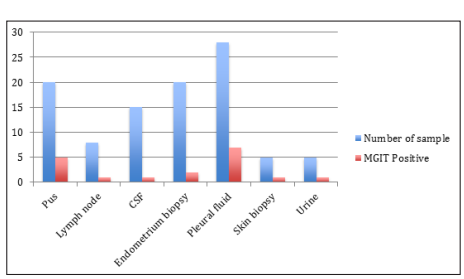

Figure 2: Figure showing total number of extrapulmonary TB suspected patients samples and MGIT-960 culture positive samples.

Discussion:

Diagnosis of EBTB is difficult due to paucibacillary nature and difficulty in getting proper sample from suspected site. As a result it is under diagnosed and may remain untreated. In present study we have detected MTB in EP samples by MGIT-960.

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In the present study ZN staining detected presence of MTB in 2 (1.98%) samples out of 101 suspected TB samples. Various other studies have also showed similar positivity by ZN staining i.e. Makeshkumar *et al* in 2014 analyzed 178 suspected EP-TB patient samples and amongst them ten (5.61%) samples were found AFB positive [18]. Similarly Maurya and co-workers in 2012 tested 756 specimens and found 9.3% (71) positivity for ZN staining [19,20]. Likewise 16% (8/50) positivity was detected by Pednekar *et al* in 2013.

In our study 17.82% EP specimens were culture positive, amongst them high positivity was observed in pleural fluid in 7 (38.89%) samples, pus in 5 (27.78%) and endometrium biopsy in 2 (11.11%) samples etc. Similarly other studies (Kandhakumari in 2015) reported 11.8% (67/570) over all positivity, highest in pus (50.75%) followed by spinal aspirate (14.93%), pleural fluid (14.93%), urine (7.46%) etc [22]. Bone and joint tuberculosis (BJTB) was reported to constitute about 10% of total extra-pulmonary TB cases by Chen *et al* using MGIT-960 [23]. Similarly Sekar *et al* reported 22% positivity for LJ culture, while 63% positivity was detected by PCR [24]. Siddiqui et al also compared MGIT culture with PCR for EP-TB diagnosis and found 5% positivity for ZN staining, 15% for both LJ culture and MGIT-960 and higher positivity of 70% for PCR [25].

Recently Gene Xpert has been used in EP-TB samples; in 2011 Zeka *et al* detected TB in 26 EP samples with 14.77% (26/176) positivity [26], Hillemann *et al* detected 8.64% positivity by analyzing 521 EP samples amongst them 45 were found positive (9.39% in tissue, 2.67% gastric fluid, 2.65% pleural fluid, 21.73% stool and 6.59 % in urine samples). [27] Sajed *et al* also detected TB in 37 EP samples (37/100; 37%), amongst them 51.7% were pus samples, 15.8% pleural fluid, 6.3% ascitic fluid and 40.0% CSF samples were found positive [28].

In present study maximum sample positivity was observed in pus (25%) and pleural fluid (25%) samples. Studies done by Amin and co-workers by PCR reported 38.6 % positivity in pus, 42.1 % in CSF and 46.6 % in urine samples, while analyzing 356 TB suspected samples [29]. While Mahesh Kumar *et al* reported 50% positivity in urine, 42.85 % in pus and 36% in CSF, 27 % in ascitic fluid, 22 % in pleural fluids by PCR and 25% in urine, 16.66% in Fine Needle Aspirate (FNA) and synovial fluid and 14.28% in pus by L-J culture [18]. Maurya and co-workers reported 83.4% positivity in lymph node aspirates and synovial fluid by MGIT-960 [19].

The mean turnaround time for detection (TTD) of mycobacteria in smear positive samples was 12.6 days and smear negative samples was 18.66 days. One study from Ambala, India reported TTD of 8 days for smear positive samples and 18 days for smear negative samples [30]. Our results also showed concordance with various other studies done on MGIT-960 showing TTD between 12-15 days [31-33].

Summary and conclusions:

As diagnosis of EP-TB is difficult due to low smear positivity and paucibacillary nature, there is need to use advanced techniques like automated liquid culture methods, PCR etc to improve the diagnosis of TB in EP samples. The present study reported *M. tuberculosis* in 17.82% cases, which is a significant, finding and demands attention. Moreover due to lack of diagnosis of TB in EP sample the incidence of drug resistance in EP samples has not been studied much. Now with availability of newer methods for detection of drug resistance in TB, it's important to develop a strategy for same as many a times the EP TB can lead to rapid deterioration in clinical condition of the patient. Use of MGIT culture could help in early detection TB and growth can be further used for detection of MDRTB.

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