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ORIGINAL RESEARCH

Comparative evaluation of detection rate and isolation of Mycobacterium tuberculosis using LJ medium, MGIT 960 and Smear microscopy from pulmonary and extrapulmonary Tuberculosis

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Abstract
Background: Tuberculosis (TB) is global public health problem, a leading cause of morbidity and mortality predominantly in developing countries. Many detection methods have been developed in the recent years. Mycobacteria Growth Indicator Tube (MGIT 960) is more precise and rapid for microbiological identification of *Mycobacterium tuberculosis* in clinical samples to prevent the spread of infection and to accelerate the effective treatment.
Aim: To compare the recovery rate and time for isolation of *Mycobacterium tuberculosis* from clinical samples by MGIT 960 and conventional Lowenstein-Jensen medium (LJ).
Materials and methods: This cross-sectional study was carried out on 80 samples which were clinically suspected cases of pulmonary and extra-pulmonary tuberculosis. Samples were processed for direct Ziehl-Neelsen staining (ZN), culture in MGIT 960 and LJ respectively.
Results: Out of 80 samples, 20 cases were positive by either of the methods. The positive samples for ZN staining, LJ medium and MGIT 960 were (12, 15%), (18, 22.5%) and (20, 25%) respectively. Among the tuberculosis positive cases, (51, 63.75%) were female and 29, 36.25%) were male, with peak incidence (43.47%) in age group of 21-40 years. The sensitivity, specificity and positive predictive value of MGIT 960 compared to LJ medium was 100%, 96.77% and 90% respectively. MGIT 960 showed faster rates of isolation (1-10 days) as compared to LJ medium (11-50 days).
Conclusion: Liquid culture medium (MGIT 960) is more accurate and rapid for the diagnosis of TB. So, it can be used in combination with LJ medium for rapid detection and early treatment of TB.
Keywords: Tuberculosis (TB), MGIT 960, Lowenstein-Jensen medium (LJ), *Mycobacterium tuberculosis.*

Introduction

Tuberculosis (TB) is an ancient disease, known to affect mankind in different forms. TB is caused by *Mycobacterium tuberculosis*, usually affects the lungs and it is a curable and preventable disease. People of both sexes and all age groups are affected by Tuberculosis with the highest burden in adult men (57% of all TB cases in 2021). About a quarter population of the entire world is assumed to expose to *Mycobacterium tuberculosis* bacteria, but most people will not manifest disease and some will clear the infection. In 2021, eight countries accounted for more than two-thirds of global TB cases: India (28%), Indonesia (9.2%), China (7.4%), Philippines (7.0%), Pakistan (5.8%), Nigeria (4.4%), Bangladesh (3.6%) and the Democratic Republic of the Congo (2.9%). People having Human Immunodeficiency Virus (HIV) are 16 to 22 times more likely to develop active TB because each infection fastens the progress of other's infection.[1] Major issues which remain a public health problem to control TB in India are poor primary health care infrastructure in rural areas of many states, spreading HIV infection, wide spread irrational use of first and second-line anti-tubercular drugs, and more recently multi-drug resistant Tuberculosis (MDR-TB).[2] The most commonly used diagnostic method to detect Mycobacteria in a clinical sample is smear microscopy, though it is rapid, cheap, easy to perform but lacks sensitivity and is not able to distinguish viable from nonviable bacteria, also it does not provide any information on drug resistance. Therefore,

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culture remains the accepted "gold standard" for diagnosing mycobacterial infections.[3] However, conventional culture on solid media like Lowenstein-Jensen media (LJ media) is cheap and simple but time-consuming, have the major disadvantage of being very slow requiring 20-56 days for diagnosis and further 4-6 weeks for drug susceptibility testing (DST), and has low sensitivity, especially in the samples containing small number of organisms.[4,5] Later on, Mycobacteria Growth Indicator Tube (MGIT 960)) system, a fully automated, high capacity and non-radiometric system has been introduced and evaluated.[6,7] The culture tube has modified Middlebrook 7H9 media with silicon-embedded fluorescent growth indicator present at the bottom of each tube. This compound is quenched by the presence of dissolved oxygen in the broth. As the microorganisms grow in the media, oxygen gets exhausted which allows fluorescence to be detected automatically over time.[8]

For the control of tuberculosis, it is necessary that pulmonary tuberculosis must be diagnosed early and treated effectively. Accordingly, recent studies showed that the MGIT culture system is a rapid, automated, and non-radiometric method, which combines with the advantage of antimicrobial susceptibility testing and bacterial identification with high sensitivity and specificity. Furthermore, the use of both the MGIT culture system and classical culture methods yields a higher reproductive rate. Therefore, this study was undertaken to compare the recovery rate and time for isolation of *Mycobacterium tuberculosis* from clinical samples by MGIT 960 vs conventional LJ media.

Materials and methods

This hospital-based cross-sectional prospective study was conducted for a period of one year from October 2020 to November 2021, after the ethical clearance from the institutional ethical committee. The proposed study had been undertaken in the Department of Microbiology, Rohilkhand Medical College and Hospital, a tertiary care hospital in the Rohilkhand region, Bareilly, Uttarpradesh. Samples were collected from clinically suspected tuberculosis cases attending departments of Pulmonary medicine based on certain inclusion and exclusion criteria as per NTEP (National Tuberculosis Elimination Programme) guidelines.

Inclusion Criteria

The study group included patients with more than any two symptoms out of the following:

- Fever with cough and expectoration ≥2 weeks
- Abnormal findings in the chest radiograph suspicious of Tubercular origin
- Gradual weight loss
- HIV status (Positive/Negative).

For extra pulmonary TB:

- Non-resolving lymph node swellings with pleural effusion and abdominal pain with low-grade fever and weight loss, etc.

Exclusion Criteria

- Cases have already on anti-tubercular drugs.
- Cases already been confirmed on tuberculosis by other microbiological methods.[9]

Total 80 pulmonary and extra-pulmonary samples were collected as per standard protocol with aseptic precautions.

Sample preparation and culture methods

All pulmonary and contaminated extra-pulmonary samples (e.g., urine, stool, or soft tissue and aspirates) were digested and decontaminated by the N acetyl-L-cysteine-NaOH procedure and processed with conventional methods for mycobacterium isolation.[5] Aseptically collected extra-pulmonary samples (e.g., blood, bone marrow, supra pubic bladder aspirates, CSF, and other body fluids, aseptically collected tissue and biopsy samples) were directly inoculated into LJ and MGIT 960 culture medium.

An equal volume of the freshly prepared MycoPrep NALC-NaOH solution was added to the sputum specimen in a sterile 50 ml conical polypropylene screw cap centrifuge tube. Tube was agitated on a vortex mixer for 30 seconds and incubated for 15 minutes at room temperature to decontaminate the specimen. The mixture was completed to double its volume with sterile phosphate buffer (pH 6.8) and centrifuged at 3000 x g for 15 minutes. The supernatant was separated and the sediment used for Acid-fast Bacilli (AFB) microscopy (ZN stain) and cultures (one solid medium LJ and one liquid medium MGIT (BACTEC MGIT 960, Becton Dickinson).

Inoculation of MGIT tubes

MGIT 960 contains 7 mL of modified Middlebrook 7H9 broth base. A lyophilized vial of BD MGIT PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin) with 15 ml of BD BACTEC MGIT OADC (Oleic acid, Albumin, Dextrose, and Catalase) was reconstituted, which is essential to suppress contamination and for the growth of many mycobacteria. The cap was unscrewed, 0.8ml of MGIT PANTA/MGIT OADC reconstituted mixture was added to make the medium complete. After that each tube was inoculated with

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0.5mL of the concentrated sample suspension prepared as mentioned above. The tube was recapped tightly and mixed well. Tubes were kept in the MGIT 960 instrument at 37°C and were monitored automatically after every hour for the increase in fluorescence for a maximum period of six weeks. Tubes which were found to be positive by the instrument, inoculated on blood agar and also, a smear was prepared to detect any contamination for the BACTEC MGIT 960 culture medium and for microscopic examination of AFB respectively. No growth was observed in the blood agar plate.

Inoculation of LJ medium

Solid LJ slant was inoculated with 0.1 mL of the suspension. Solid medium was incubated at 37°C in a slant position for 24-48 hours, then in the upright position for a further eight weeks, and read daily for the 1st week then twice weekly. Colonies of *Mycobacterium tuberculosis* were identified by their rough, crumbly, waxy, buff-coloured appearance which developed after 2-3 weeks after inoculation. The colonies with doubtful morphology were confirmed by ZN staining.[10]

Statistical Analysis

Descriptive analysis was done. Data was arranged into numbers and percentages.

Results

Total 80, pulmonary and extrapulmonary samples were processed using ZN stain, LJ media, and MGIT 960. Out of 80 samples, 25 (31.25%) were pulmonary including sputum (19, 23.75%), bronchoalveolar lavage (6, 7.5%) and 55 (68.75%) were extrapulmonary including pus (14, 17.5%), gastric aspirate (13, 16.25%), endometrial tissue (7, 8.75%), soft tissue (6, 7.5%), pleural fluid (11, 13.75%), biopsy fluid (1, 1.25%) and synovial fluid (3, 3.75%) samples.[Table1] The majority of cases (46, 57.5%) were found in 21-40 years of age group, while (21, 26.25%) in 41-60 years.[Table 2] In our study, 51 (63.75%) samples were from female and 29 (36.25%) from male patients.[Table 3] Out of total 80 samples under study, 20 samples were found positive by any of the three methods viz., ZN Stain, LJ Medium or MGIT 960. Among 20 positive samples, 18 (22.5%) were found positive by LJ medium culture and 12 (15%) by ZN staining but MGIT 960 detected all 20 (25%) samples. [Table 4] Distribution of samples according to culture positivity by different culture methods employed are shown in Table 5 in tabulated form. The sensitivity, specificity, and positive predictive value of MGIT 960 compared to LJ medium was 100%, 96.77% and 90% respectively. [Table 6] Out of 20 positive samples, 12 were found positive within 10 days and 8 showed positivity within 11-20 days by MGIT 960. On the other hand, we found that out of 18 samples detected by LJ medium, 4 showed positivity in 11-20 days and another 4 in 31-40 days. Next 10 samples showed the highest duration of positivity in 41-50 days as shown in Table 7.

Table1. Types of samples

SN	Sample	Number
1.	Sputum	19 (23.75%)
2.	Brochoalveolar lavage	6 (7.5%)
3.	Pus	14 (17.5%)
4.	Gastric aspirate	13 (16.25%)
5.	Endometrial tissue	7 (8.75%)
6.	Tissue	6 (7.5%)
7.	Pleural fluid	11 (13.75%)
8.	Biopsy tissue	1 (1.25%)
9.	Synovial fluid	3 (3.75%)
	Total (n=80)	80

Table 2: Distribution of samples according to age and site

Age in years	Pulmonary	Extrapulmonary	Total
1-20	5 (20%	8 (14.54%)	13(16.25%)
21-40	12 (48%)	34 (61.81%)	46 (57.5%)
41-60	8 (32%))	13 (23.63%)	21 (26.25%)
Total (n=80)	25	55	80

Table 3: Distribution of samples according to sex

Age in years	Males	Females	Total
1-20	6	7	13 (16.25%)
21-40	15	31	46 (57.5%)

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41-60	8	13	21(26.25%)
Total (n=80)	29 (36.25%)	51 (63.75%)	80

Table 4: Distribution of test results as per the different diagnostic test

Diagnostic Test	Positive Samples	Negative Samples	Total (n=80)
ZN Stain	12	68	80
LJ Media	18	62	80
MGIT 960	20	60	80

Table 5: Distribution of samples according to culture positivity

Sample Type and their number	LJ medium positive	LJ medium negative	MGIT Positive	MGIT Negative	Direct smear positive	Direct smear Negative
Sputum (n=19)	8 (44.44 %)	11(55.56%)	8 (40%)	11 (60%)	5 (26.31%)	14 (73.68%)
BAL (n=6)	1 (16.67%)	5(83.34%)	1 (16.67%)	5 (83.34%)	1 (16.67%)	5 (83.34%)
Gastric aspirate (n=13)	2 (15.38%)	11(84.62%)	2 (15.38%)	11 (84.62%)	2 (15.38%)	11 (84.61%)
Endometrial tissue (n=7)	1 (14.28%)	6 (85.72%)	1 (14.28%)	6 (85.72%)	1 (14.28%)	6 (85.72%)
Soft Tissue (n=6)	0	6 (100%)	0	6 (100%)	0	6 (100%)
Pus (n=14)	2(14.28%)	12(85.72%)	3 (21.42%)	11 (78.58%)	2 (14.28%)	12 (85.71%)
Pleural fluid (n=11)	3 (27.27%)	8 (72.73%)	4 (36.36%)	7 (63.64%)	1 (9.09%)	10 (90.90%)
Biopsy fluid (n=1)	0	1 (100%)	0	1 (100%)	0	1 (100%)
Synovial fluid (n=3)	1 (33.33%)	2 (66.67%)	1 (33.33%)	2 (66.67%)	0	3(100%)
Total (n=80)	18 (22.5%)	62 (77.5%)	20 (25%)	60 (75%)	12 (15%)	68 (85%)

Table 6: Sensitivity, Specificity and Positive predictive value of MGIT 9 6 0 to LJ medium culture

	LJ medium positive	LJ medium negative	Total
MGIT positive	18 (90%)	2(10%)	20
MGIT negative	00	60	60
Total (n=80)	18	62	80

Sensitivity	100%
Specificity	96.77%
Positive predictive value	90%

Table 7: Duration of isolation of MGIT 960 and LJ medium.

Number of days	No. of positive MGIT	No. of positive LJ
1-10	12	0
11-20	8	4
21-30	0	0
31-40	0	4
41-50	0	10
51-60	0	0
Total	20	18

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Figure 1: Showing growth on LJ medium- rough, tough and buff colored colony

Figure 2: Showing growth in MGIT tube- Flaky growth in liquid medium.

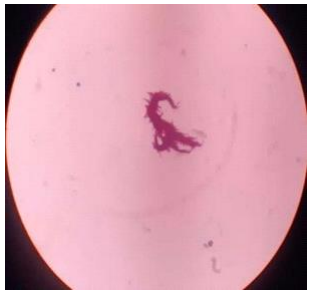

Figure 3: AFB smear from MGIT tube showing cording phenomenon (cording is a complex phenotype in which many AFB arranged in parallel chains)

Discussion

In India, TB is a major global public health problem. The early detection of tuberculosis helps in initial treatment, and thus prevents the possible transmission of the infection. A delay in diagnosis may aggravate the disease, enhances transmission, and increase the mortality rate.[11]

We compared the detection rate and isolation of *Mycobacterium tuberculosis* using LJ media, MGIT 960 and smear microscopy from samples taken from suspected pulmonary and extrapulmonary tuberculosis patients. The study included 80 clinically suspected cases of both pulmonary and extrapulmonary tuberculosis. Out of 80 samples (25, 31.25%) were pulmonary including sputum and bronchoalveolar lavage samples and (55, 68.75%) were extrapulmonary including pus, gastric aspirate, endometrial tissue, soft tissue, pleural fluid, biopsy and synovial fluid.

In our study, we observed female predominance (51, 63.75%) over male (29, 36.25%) whereas in other studies, higher incidence of tuberculosis was found among males. [12,13,14] This could be due to females having increased outdoor activity and wide social network now a days. Moreover, the incidence of infertility is also increased among the young female population and tuberculosis is one of the major causes of it.

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We found a maximum number of suspected cases in 21-40 years of age group; similar results were also seen by Zhang et. al. [15] whereas the study conducted by Gopi A et. al. [16] showed that patients in 41-50 age group had a high prevalence.

Though the pulmonary form is the most common presentation, extrapulmonary tuberculosis is also an important emerging clinical problem. [17] Extrapulmonary tubercular infections are more often smear negative then pulmonary cases which makes its diagnosis difficult to establish.[18] The diagnosis of extrapulmonary tuberculosis is not always promising with conventional methods, due to the longer time required for the cultivation and the paucibacillary nature of samples. [19] Therefore for a definitive diagnosis, an array of manual and automated systems has been developed to trim down the time to detect and identify bacteria in the clinical samples. [20]

Smear microscopy for acid-fast bacilli (AFB) is most commonly used diagnostic test for tuberculosis in developing countries. In our study out of 80, (12 ,15%) samples were found positive by smear microscopy which is almost similar to the study conducted by others [21,22,23,24], while Roohi Aftab et. al. [25] and Salam AA et. al. [26] found lower results; 10.3% and 11.33% respectively with smear microscopy.

The direct smear positivity rate is higher for respiratory tract specimens compared to others. The factors affecting sensitivity are: the quality of the specimen, loss during centrifugation, the staining and culture method and the case group evaluated. Sometimes the detection of acid-fast bacilli from smear microscopy is not possible, so in that case culture on solid medium is the best way to rule out the missed diagnosis from the smear microscopy. [27,28]

The vast majority of tubercular cases go undiagnosed in developing countries due to the non-availability of appropriate laboratory facilities. Solid culture medium is cheaper and more widely available, but is labour-intensive and slower than liquid culture. [29,30,31] While liquid medium is known to be more sensitive and rapid for the isolation of mycobacteria. Most laboratories in developing countries still rely on solid egg-based LJ media for *Mycobacterium tuberculosis*. CDC recommends use of both liquid and solid medium for maximum recovery of *Mycobacterium tuberculosis*. [32,33]

Unlike the solid medium, the liquid medium has the advantage of recovering the bacteria present in the sample even at a reduced number, but also the bacteria stressed by the treatment preceding the culture.[34] Nevertheless, some other studies found no significant difference between the two methods. [35,36,37]

In our study out of 80 samples, 20 (25%) were found positive for *Mycobacterium tuberculosis* by MGIT 960. In a study conducted by Anjana Gopi et. al. [16] found 12/100 (12%) positive samples by MGIT 960.

When we compared MGIT 960 with LJ medium, out of 20 positive samples 2 were not detected by LJ medium. The present study demonstrated that MGIT 960 system 20 (25%) provides better isolation and detection rate of *Mycobacterium tuberculosis* from a variety of clinical samples compared to solid media 18 (22.5%). This is due to bigger size of inoculum used and partly due to addition of nutritional supplements in MGIT culture tubes.

The sensitivity, specificity, and positive predictive value of MGIT 960 compared to LJ media was 100%, 96.77%, and 90% respectively similar to U Pratibha Bhat et.al. [13] 100%, 97.5%, and 91.3% respectively.

In our study, we observed that the time to detection of *Mycobacterium tuberculosis* with the MGIT 960 was shorter than that with the LJ medium. The turn-around time in our study for the MGIT 960 was 1 to 20 days and for LJ medium was 11-50 days. Our study was in accordance with T.S. Essawy et. al. [38] who found the mean time for detection for MGIT 960 was average 10.7 ranges from 5 to 20 days. Rivera et. al. [37] also found that the mean time for detection of positive culture by MGIT 960 was shorter than LJ medium. In the study done by Vahishth Mishra et.al. [39], turn-around time for the MGIT 960 system was 3 to 40 days and for LJ media 8 to 42 days.

MGIT 960 culture media is a rapid and more sensitive diagnostic test for *Mycobacterium tuberculosis* than the traditional ZN stain and LJ culture media. The combination of both MGIT 960 and LJ media enhances the recovery of *Mycobacterium tuberculosis* from clinical samples.

Conclusion

Liquid culture (MGIT 960) is more accurate and better than LJ media, ZN in recovery rate and detection time of mycobacterial growth. Early and appropriate diagnosis of TB may result in appropriate use of anti-tubercular drugs, and prevent the emergence of multidrug resistant tuberculosis (MDR-TB). Thus reduces the overall morbidity and mortality due to the disease.

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