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ORIGINAL RESEARCH

Performance of Mycobacterium Growth Indicator Tube (MGIT 960) for detection of Mycobacterium Tuberculosis

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ABSTRACT

Background: Despite advances, early and accurate tuberculosis (TB) diagnosis remains challenging, particularly in high-burden regions like India.Apart from the conventional methods, Mycobacterium Growth Indicator Tube (MGIT 960), a non-radiometric liquid culture, has rapid turnover and a good recovery rate. The use of MGIT is still limited due to high burden on resources. This study is designed to assess the performance of MGIT in comparison toother tests for the detection of TB. **Methods:** A prospective observational study was conducted on 778 pulmonary samples collected between January 2023 and June 2024 at a tertiary care center in North India, using microscopy, solid culture (Lowenstein-Jensen medium), liquid culture (MGIT 960), and the CBNAAT. The samples were processed using standard procedure and manufacturer's protocol. Data was analyzed using SPSS 23.0. The sensitivity, specificity, predictive value, and average turnaround time were calculated. **Results:** Out of 778 samples, 223 (28.7%), isolates were detected MTB by MGIT, followed by 216(27.8%) isolates from CBNAAT along with 3 errors, 190 (24.4%) by Solid culture along with 7 contaminated culture and 85 (10.9%) isolates by microscopy. The MTB is detected significantly faster in MGIT cultures than in LJ cultures. The agreement between MGIT 960, solid culture and CBNAAT was high (Kappa= 0.891, Kappa= 0.978 respectively). **Conclusion:** MGIT is an automated method and provides quicker results than conventional methods like microscopy and solid culture. The results are comparable to other conventional tests includingCBNAAT.The findings underscore the importance of advanced diagnostic tools in improving TB management and reducing detection delays, particularly in regions with limited resources.

INTRODUCTION

Tuberculosis (TB) is a preventable and usually curable disease. One-fourth population of the world which is two billion people may be infected with TB, with 10.6 million getting sick every year.[1] In India, 7.3 lakh cases has been notified so far, despite intense surveillance under the National TB Elimination Program (NTEP). The World Health Organization (WHO) has projected that inadequate surveillance and delayed diagnosis could resultin approximately 75 million people developing drug-resistant (DR) TB, resulting in an economic loss of $16.7 trillion globally in the next 35 years.[2]These numbers may not reflect the exact projection due to the in-between COVID-19 pandemic.

For many years, the World Health Organization (WHO) has employed a straightforward and practical approach by offering standardized drug regimens for TB treatment worldwide. However, addressing the increasing challenge of the TB epidemic requires universal access to genotypic testing and tailored treatment protocols. Advanced and accessible diagnostic tools are essential for effectively reducing the global TB burden.The diagnosis of TB relies primarily on bacteriologically confirmed cases. In many developing countries, conventional methods like sputum microscopy using the Ziehl-Neelsen

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technique are widely used. However, this method has low sensitivity, with a detection limit of 10,000 bacilli per milliliter of sputum, identifying only 10-75% of cases.[3]The Lowenstein-Jensen medium can detect 10–100 bacilli per milliliter of sputum. However, it has significant limitations, including a lengthy processing time of 3–8 weeks, low sensitivity, and an inability to distinguish Mycobacterium tuberculosis (MTB) from other acid-fast bacilli.The non-radiometric liquid culture which is known as*Mycobacterium* Growth Indicator Tube (MGIT 960) has a better yield of MTB and in addition, it is automatic, and fully radiometric. The system can simultaneously process and monitor up to 960 samples with an automated result-reporting feature. Nucleic acid amplification tests (NAATs), such as GeneXpert (Xpert MTB/RIF) and Xpert MTB/RIF Ultra, are highly sensitive and specific tools for detecting MTB in sputum samples and are now the preferred diagnostic tests for TB. The advent and implementation of NAATs have revolutionized TB diagnostics by enabling the detection of M. tuberculosis DNA directly in clinical specimens. However, their effectiveness largely depends on the quality of the sample being tested. Despite these advancements, achieving timely and accurate TB diagnosis remains a considerable challenge, particularly in high-burden countries like India.It can incubate and monitor 960 samples simultaneously with an automated result-reporting system. Nucleic acid amplification tests (NAATs), such as Gene Xpert (Xpert MTB/RIF) and Xpert MTB/RIF Ultra, provide highly sensitive and specific detection of MTB in sputum samples and serve as the primary tests for TB diagnosis. The development and adoption of NAATs have transformed the field of TB. By detecting MTB DNA in samples, their effectiveness is closely linked to the quality of the specimen analyzed. Despite the availability of advanced tools and techniques, early and accurate diagnosis of TB remains a significant challenge, especially in high-burden countries like India. This study was conducted to assess the diagnostic efficacy of MGIT 960 in comparison with other conventional methods and CBNAAT, in addition to the burden of pulmonary TB casesin northern India.

METHODS

Study design and setting

This prospective observational study was conducted between January 2023 and 30 June 2024 in Mycobacteriology Culture and DST laboratory Uttar Pradesh University of Medical Sciences, Saifai, Etawah, UP. India, a tertiary care medical college in North India. The study was approved by institutional ethics committee (69/2022-23) and written informed consent was obtained from all the subjects.

Inclusion and Exclusion criteria

Suspected pulmonary TB patients visiting out patient departments (OPDs) and in-patient department (IPDs) of different clinical departments fulfilling inclusion criteria were included in the study. The inclusion criteria were TB like symptoms having cough >2 weeks, fever >2 weeks, significant weight loss, haemoptysis or abnormalities in chest radiography with no improvement during seven to ten days or household contact infected with TB/DR -TB within the previous 3 months. The new TB patient or Patient non-responder to treatment. The patients who refused to take part in the study or had insufficient sample for processing were excluded from the study.

Sample collection

After enrolment, history and examination were performed. Relevant investigations were noted and recorded in the patient record proforma. A total of 778 pulmonary isolates were obtained and analyzed during the study period. All the samples were processed within a Biosafety Level 3 (BSL-3) to avoid the exposure risk from aerosol production. Upon receipt in the laboratory, all samples were divided into three portions. One portion was sent for microscopy, second for culture and third for the CBNAAT assay. Direct smears were prepared from specimens using Ziehl-Neelsen staining. Non-sterile clinical samples were processed with the N-acetyl-L-cysteine-sodium citrate-NaOH method. After centrifugation, the samples were decanted, and the sediments were resuspended in 3 mL of phosphate-buffered solution. The processed samples were then inoculated on either Lowenstein-Jensen solid medium or BACTEC liquid culture. MGIT was performed as per the manufacturer's protocol ((BACTEC MGIT 960 (Becton Dickinson, Sparks, MD, USA)). The CBNAAT was conducted following the manufacturer's instructions (GENE XPERT MTB/RIF Cepheid, Sunnyvale, CA, USA)®. TB Ag MPT64 Rapid test was put on all these positive cultures.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences), Version 23.0. Categorical variables were described with frequencies and percentages, while numerical variables were reported as means with standard deviations (SD). Sensitivity, specificity, and positive and negative predictive values were calculated to evaluate the comparative performance of the tests. The agreement between tests was assessed using Cohen's Kappa statistic. A significance level of $P \leq 0.05$ was set for statistical analyses. Estimates were presented with 95% confidence intervals (95% CI) to indicate precision.

RESULTS

A total of 778 pulmonary isolates were analysed during the study period. The majority of the samples were sputum accounting for 700 cases (90.0%) followed by gastric aspirate, which contributed 45 cases (5.8%), and BAL samples, which was 33 cases (4.2%).

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Out of 778, 223 (28.66%) were MTB positive by any of the above methods, and the respective prevalence as per type of sample is depicted in fig1. Most patients fall into the 21-40-year age group, comprising 39.0% of the study population. The mean age of the patients is 34.64 ±19.1 years, reflecting a broad age range from 1 to 87 years. Males constituted the majority of the study population, with 121 (54.3%) individuals, while females numbered 102 (45.7%).

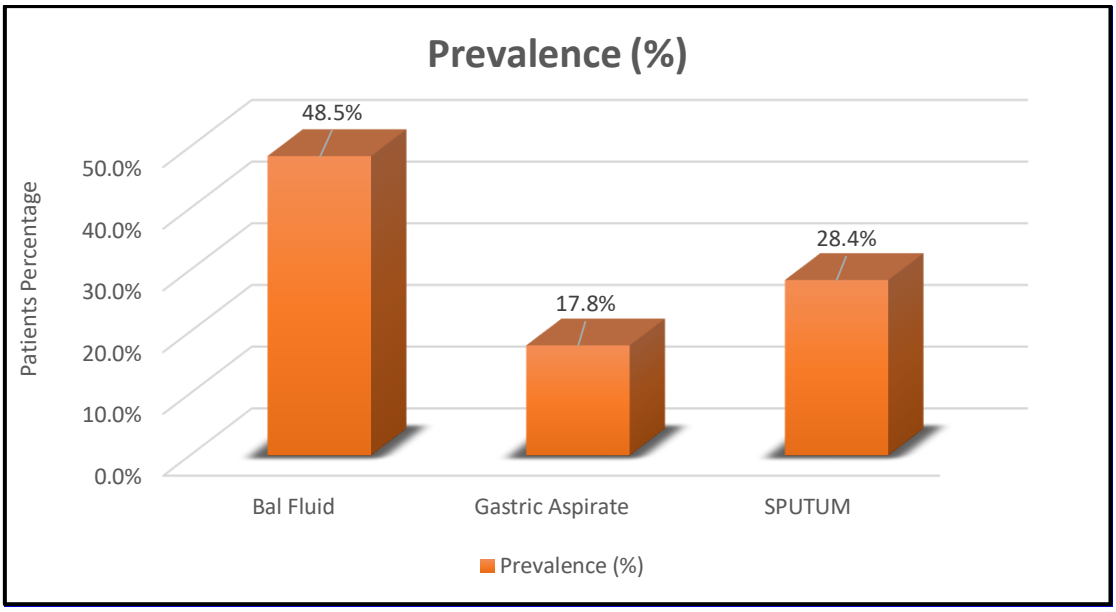

Fig. 1: Prevalence of the MTB detection in the total study isolates

Out of 778 samples, 223 (28.7%), isolates were detected MTB by MGIT, followed by 216(27.8%) isolates from CBNAAT along with 3 errors, 190 (24.4%) by Solid culture along with 7 contaminated culture and 85 (10.9%) isolates by microscopy. (Fig 2)

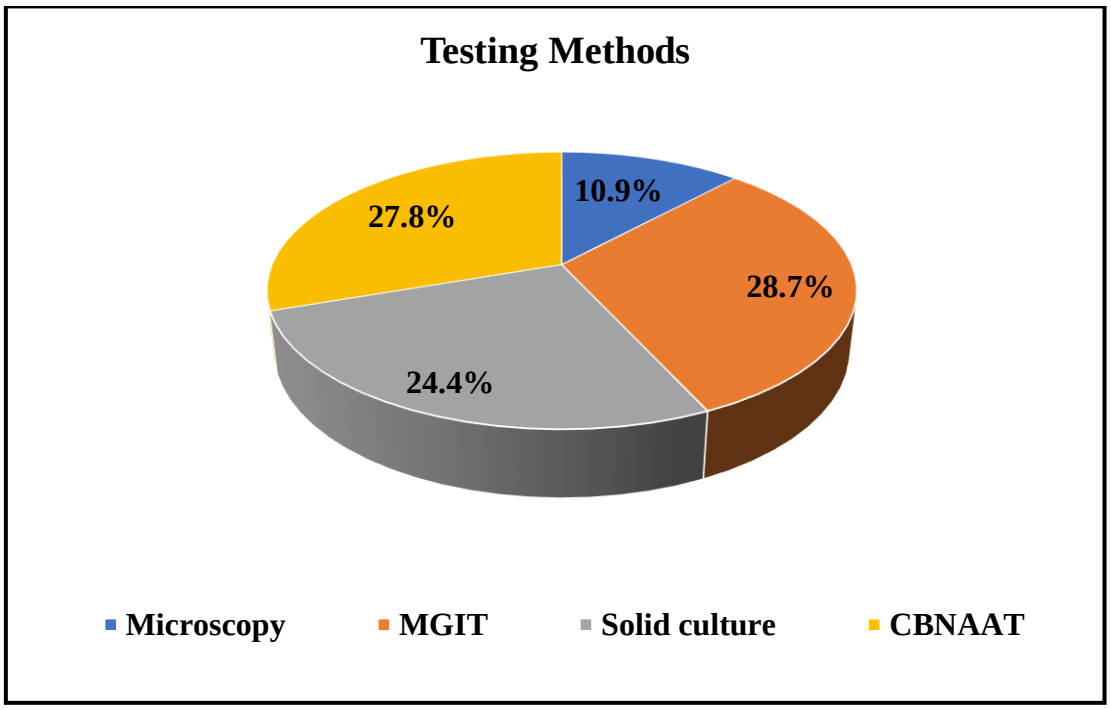

Fig. 2: Distribution of MTB-positive isolates based on different testing methods

Table 1 presents a comparative analysis of MTB positivity across different types of samples and diagnostic tests. The data reveals that BAL samples yielded the highest number of positive results among the various sample types across all diagnostic methods.

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Table 1: Comparative detection of MTB positivity according to the type of sample and various tests

Nature of the Sample	MICROSCOPY		MGIT/ MPT 64 KIT		SOLID CULTURE		CBNAAT	
	Positive n(%)	Negative n(%)	Positive n(%)	Negative n(%)	Positive n(%)	Negative n (%)	Positive n(%)	Negative n(%)
BAL Fluid (n=33)	5 (15.1)	28 (84.8)	16(48.4)	17(51.5)	9 (27.2)	24(72.7)	10 (30.3)	23 (69.6)
Gastric Aspirate (n=45)	3 (6.6)	42 (93.3)	8 (17.7)	37 (82.2)	5(11.1)	40(88.8)	5 (11.1)	40(88.8)
Sputum (n=700)	77 (11)	623(89.0)	199 (28.4)	501 (71.5)	176 (25.1)	524(74.8)	201 (28.7)	499(71.3)
Total (n=778)	85 (10.9)	693 (89.1)	223 (28.6)	555 (71.4)	190 (24.4)	588(75.6)	216 (27.7)	562 (72.2)

The MTB is detected significantly faster in MGIT cultures than in LJ cultures in all the samples. The mean TTD in BAL, GA and sputum samples for the MGIT method was 19.2± 6.91 days, 17.9±6.4 days and 18.8±6.8 days respectively. On the contrary, The solid culture method showed a mean TTD of 35.2±6.3 days, 32.5±4.1 days, 34.4±6.1 days in BAL, GA and sputum samples respectively. Table 2 presents a comparative analysis of the performance of microscopy, solid culture, and CBNAAT against the MGIT method for the detection of MTB.The p-value of <0.001 indicates that this difference is statistically highly significant.

Table 2: Performance of Microscopy, solid culture, and CBNAAT in comparison to MGIT for the detection of MTB

		MGIT		TOTAL			95% confidence interval (%)
					p-value	<0.001	
					Kappa value	0.468	0.4-0.5
					Sensitivity	38.12%	31.7-44.8
		Positive	Negative		Specificity	100.0%	99.3-100.0
Microscopy	Positive	85	0	85	PPV	100.0%	95.7-100.0
	Negative	138	555	693	NPV	80.1%	78.3-81.6
Total		223	555	778	Accuracy	82.2%	79.3-84.8
		MGIT		Total	p-value	<0.001	
					Kappa value	0.891	0.85-0.92
					Sensitivity	85.20%	79.85-89.5
		Positive	Negative		Specificity	100%	99.34-100
Solid Culture	Positive	190	0	190	PPV	100.0%	98.08-100
	Negative	33	555	33	NPV	94.39%	92.47-95.48
Total		223	555	778	Accuracy	95.76%	94.09-97.06
		MGIT		Total	p-value	<0.001	
					Kappa value	0.978	0.96-0.99
					Sensitivity	96.86%	93.64-98.73
		Positive	Negative		Specificity	100.0%	99.34-100
CBNAAT	Positive	216	0	216	PPV	100.0%	98.31-100
	Negative	7	555	562	NPV	98.75%	97.45-99.4
Total		223	555	778	Accuracy	99.10%	98.16-99.64

While microscopy has limited sensitivity and moderate overall performance, solid culture and CBNAAT show significantly improved detection capabilities. CBNAAT, in particular, provides reliable results with excellent sensitivity, specificity, and overall accuracy.

DISCUSSION

TB is a global threat and emerging drug resistance to TB is particularly alarming. There are various modalities available to detect TB. With the introduction ofmethods like MGIT or Xpert/RIF assay for the rapid diagnosis of TB, there has been a significant reduction in time to initiation of treatment in TB suspect cases.[4] The WHO recommends the increasing use of recommended molecular diagnostic methods like GeneXpert and MGIT to improve their accessibility to increase the percentage of MTB diagnoses as well as to detect drug resistance patterns

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to achieve earlier and more accurate diagnosis and treatment of TB. The current study has prospectively evaluated the burden of the disease and performance of MGIT over other conventional tests in MTB-suspected patients as only a few studies are available from North India.

In our study, we evaluated 778 isolates suspected for pulmonary TB out of which 223 isolates were positive for MTB and were evaluated for resistance profile of MTB. These 223 isolates were enrolled based on MGIT MTB positive results and this test was taken as a standard test for MTB detection and drug resistance. The mean age in our study was 34.64 years with the maximum affected patients from the age group of 21-40 years (39%) which was almost similar to the study conducted by **Misra *et al.* and Sinha *et al.*** where the maximum affected patients belonged the age group 21-45 years.[5,6] Our cohort was dominated by males (54.3%) as compared to females (45.7%) which was similar to the study by **Mishra *et al*. and Giri *et al*.** observing male preponderance of 49.2%.[7,8]The high frequency of the disease among the younger population may facilitate the transmission of TB in the community due to the greater mobility of youth. A gender analysis of the TB epidemic shows that TB affects different genders differently. Studies have shown that women may be diagnosed late or not diagnosed at all due to socio-cultural barriers such as a high burden of household work, illiteracy, restricted mobility as well as lack of autonomy.[9]

Out of the 778 samples, 223 samples were detected for MTB (28.7%) either by microscopy, solid culture (LJ), MGIT or CBNAAT. **Kanade *et al*.** also observed comparable results where the detection rate was 27.74%.[10] **Diriba *et al*.** also reported 26% MTB in their study which was comparable to our study.[11] In our study, diagnostic yield for microscopy, MGIT960, solid culture and CBNAAT were 10.9%, 28.6%, 24.4% and 27.7% respectively. The results of **Gopi *et al*.** on the contrary had a lower diagnostic yield ofmicroscopy, MGIT960, and solid culture at 4%, 12%, and 6% respectively.[12]***Sharmaet al*.** observed that out of 8123 samples, 508 (6.2%) specimens were positive by MGIT, 371 (4.6%) by Gene Xpert.MGIT detected 137 (1.7%) extra positive than GeneXpert.Good sensitivity (73%) and concordance (96.8%) were observed for GeneXpert against MGIT culture in this study[13]In our study, we reported more positive cases in comparison to the above studies the reason could be more sample load and high prevalence of TB in our area.

In our study, TTD for MTB in liquid culture (MGIT 960) in all the samples was significantly less than solid culture. The study by **Lee *et al.*** reported the mean TTD of MTB complex as 11.6 days with MGIT 960 and 20.1 days with LJ.[14]Comparable TTD for smear-positive specimens was reported from Yugoslavia and India with average TTD time for BACTEC MGIT and LJ method of 13.7 days and 22.1 days; 13.1 and 23.9 days respectively.[15,16] Thus, in the present study, BACTEC MGIT 960 was found to be more rapid and efficient than that of the solid media. Rapidity and the higher sensitivity of the MGIT 960 system will play a role in the recovery of mycobacteria from samples.***Risso et al***in a retrospective study on the cohort of pulmonary tuberculosis observed a positive correlation between contact positivity. The transmission rate was 44% when the TTD was <9 days and only 22% when TTD was >9 days.[17]

In the present study, the sensitivity, specificity, PPV, NPV and accuracy of microscopy as compared to MGIT were 38.12%, 100%, 100%, 80.1% and 82.2%. Our study results were contrary to the study by **Rattan *et al*.** who examined a total of 1,520 samples and observed the overall sensitivity, specificity, PPV, and NPV of, 83.72, 91.91, 71.38, and 95.91% respectively. [18]**Rachow *et al*.** alsoobserved 30.9% positivity by sputum smear microscopy, resulting in a sensitivity of 72.9% (43/59; 95%CI: 59.7–83.6) in comparison to culture.[19]The sensitivity of microscopy in our study was lower than in the literature. The overall clinical sensitivity depends on the burden of mycobacterium load in the sample, the staining technique and the experienced laboratory technicians. Hence the results of microscopy are dependent on many factors so the sensitivity of microscopy in our study was lower. In our study, the sensitivity of LJ media in comparison to MGIT was to the tune of 85.20% which was in concordance with the studies available in the literature. **Mishra *et al*.** obtained a fair sensitivity of liquid culture, amongst the total positive specimens, 94% were detected by MGIT and 89% were positive with LJ. CBNAAT sensitivity, specificity, PPV, and NPV, were 96.94, 81.22, 55.42, and 99.10% respectively.[7] A recent 2019 systematic review which included 59 studies assessing the accuracy of molecular diagnostic methods for the detection of pulmonary TB was performed in China. The highest pooled sensitivity was from Xpert MTB/RIF (20 studies; pooled sensitivity 91%, 95% CI 87–94%).[20] The comparative analysis of CBNAAT and MGIT showed very high sensitivity and specificity of 96.86% and 100% respectively. The agreement value between the two test methods was also very high (k=0.978). Similar findings were obtained in the study by **Kanade *et al*.** where the sensitivity and specificity of the Gene Xpert assay compared to culture were 92.1% and 92.6%, respectively.[10]Another study by **Misra *et al.*** also observed an 86.6% agreement between MGIT culture and Xpert assay.[5] This variability in diagnostic accuracy may be affected by the bacteriological burden in the sample.

ABBREVIATIONS LIST

AFB – Acid Fast Bacilli
ATT – Anti-tubercular Treatment
BSL- Bio Safety Lab

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CBNAAT – Cartridge-Based Nucleic Acid Amplification Test
DNA – Deoxyribose Nucleic Acid
DOTS – Directly Observed Therapy –short course
DST – Drug Susceptibility Test
EPTB – Extra-Pulmonary TB
FL-LPA – First Line -Line Probe Assay
HIV – Human Immunodeficiency Virus
LJ – Lowenstein Jensen LPA – Line Probe Assay
MB – Middlebrook
MDR-TB – Multi-Drug Resistant –TB
MGIT – Mycobacterial Growth Indicator Tube
MIC – Minimum Inhibitory Concentration
MTB – *Mycobacterium tuberculosis*
NAAT- Nucleic acid amplification test
NALC – N-Acetyl L-Cysteine
NaOH – Sodium Hydroxide
NTEP – National TB Elimination Programme
PCR – Polymerase Chain Reaction
PTB – Pulmonary TB
RNTCP – Revised National TB Control Programme
SSC – Standard Sodium Citrate
TTD – Time To Detection
TST – Tuberculin Skin Test
WHO – World Health Organization
XDR-TB – Extensively Drug Resistant-TB
Xpert MTB/RIF-
ZN – Ziehl-Neelsen

CONCLUSIONS

Our findings indicate that the BACTEC MGIT liquid culture system outperforms conventional LJ methods, providing faster recovery of the *M. tuberculosis* complex with a significantly shorter turnaround time. The liquid culture method demonstrated a shorter mean time to detection, and its process can be automated, enabling efficient handling of large volumes of specimens.

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