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Research Article

Comparison of MGIT960 with Lowenstein-Jensen Method for *Mycobacteria* Detection in Pulmonary Tuberculosis and Drug Susceptibility Testing

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Abstract

Introduction: This study aimed to evaluate the performance of Mycobacterium Growth Indicator Tube 960 (MGIT960) and Lowenstein-Jensen (LJ) medium for recovery of mycobacteria in pulmonary tuberculosis (PTB) and the drug susceptibility testing (DST).

Materials and Methods: A total of 2411 pulmonary specimens were collected from TB suspected patients during the study period (July 2018 to March 2020). Specimens were inoculated into both MGIT960 and LJ medium followed by Ziehl-Neelsen (ZN) and Auramine Phenol (AP) staining. DST was performed in MGIT 960 and LJ medium.

Results: Of 2411 pulmonary specimens, 190 (7.0%) and 153 (5.7%) were culture positive by MGIT960 and LJ, respectively. Of the 190 MGIT positive cultures, 169 (88.9%) were Mycobacterium tuberculosis complex (MTB) and 21 (11.1%) were non-tuberculous mycobacteria (NTM). From 153 LJ positive cultures, 138 (90.2%) and 15 (9.8%) were identified as MTB and NTM, respectively. Contamination rate associated with MGIT960 and LJ culture was 125 (5.2%) and 113 (4.7%), respectively. The average time to detection for MTB in MGIT960 and LJ were 18.17 ± 8.69 and 32.46 ± 10.46 days, respectively (p value < 0.0001) and for NTM was 18.76 ± 10.67 and 21.00 ± 12.12 days, respectively (p value 0.5611). Sensitivity of streptomycin (S), isoniazid (I), rifampicin (R) and ethambutol (E) were 87.5%, 100%, 100% and 90.9%, respectively. Specificity of S, I, R, E were 99.3%, 100%, 100% and 98.6%, respectively

Conclusion: MGIT960 has better recovery, with shorter time to detection and similar contamination rate in pulmonary specimens compared to LJ method. The DST performance of MGIT960 was comparable to LJ.

Keywords: Tuberculosis; MGIT960; LJ Medium; Contamination Rate; Drug Susceptibility Testing

Introduction

Pulmonary tuberculosis (PTB) due to *Mycobacterium tuberculosis* (MTB) and non-tuberculous mycobacteria (NTM) causes significant mortality and morbidity to the patients [1]. Prevalence of tuberculosis (TB) was estimated to be 13 million worldwide and India accounts for nearly one fourth of the above burden. WHO report 2020, reported India to be a highest TB burden country globally and was estimated to have an incidence of 26.9 lakh cases in 2019 [2]. Prevalence of non-tuberculous mycobacteria (NTM) raised from 2.4 to 15.2 cases per 100,000 in US in 2013, from 4.65 cases to 9.08 cases per 100,000 in Canada in 2010 [3]. In North Indian study prevalence of NTM was estimated to be 0.77% and the isolation rate of NTM has increased from 0.5% to 8.6% [4,5].

Since the prevalence of both MTB and NTM are on the rise, it is essential to identify these pathogens by using a rapid and more sensitive method and start the patients on the appropriate antimycobacterials as early as possible [6]. The poor sensitivity of smear microscopy and prolonged duration for mycobacterial identification by solid Lowenstein Jensen (LJ) culture are the major limitations of conventional methods [7]. Rapid molecular methods such as GeneXpert and Truenat are relatively expensive and their use in resource-limited settings is difficult [8]. Center for Disease Control and Prevention (CDC) and World Health Organization (WHO) recommends the use of liquid culture as the gold standard method for both primary isolation and drug susceptibility testing (DST) of MTB [9]. *Mycobacterium* Growth Indicator Tube (MGIT; Becton

Dickinson), a non-radiometric method is a fully automated system helps in the better recovery of *Mycobacterium*. But the disadvantage of them is the high rates of contamination that was reported [10]. In this context, we aimed to compare the performance of BACTEC MGIT 960 with LJ method for detection of Mycobacteria in clinically suspected TB patients and to compare the results of drug susceptibility testing using MGIT 960 and LJ medium.

Materials and Methods

We conducted this prospective hospital-based study after obtaining Institutes Ethics Committee approval in the department of Microbiology from July 2018 to March 2020. The study included 2411 pulmonary specimens (sputum, tracheal aspirate, endotracheal aspirate, gastric aspirate) from the suspected tuberculosis patients.

Sample processing

The clinical pulmonary specimens were processed as per the Revised National Tuberculosis Control Programme (RNTCP) guidelines followed in India. The samples were decontaminated by standard N-Acetyl L-cysteine (NALC)/Sodium Hydroxide (NaOH) method. The decontaminated samples were subjected to Ziehl Neelsen (ZN) or Auramine Phenol (AP) staining and two to three drops were inoculated into LJ medium and 0.5 ml of the specimen was inoculated in MGIT 960 tubes. Inoculated LJ medium was incubated at 37°C for 8 weeks and the MGIT 960 tubes were incubated in the MGIT960 system (BD Bactec™ MGIT™ 960, BD Biosciences, Mumbai, Catalog # 445870) till the tubes were flagged positive by the machine or till it was negative up to 42 days. The LJ slants were inspected for the presence of typical Mycobacterial colonies twice a week. Once the growth was seen on LJ or the MGIT 960 was flagged positive they were subjected to ZN staining and was subcultured in 5% sheep blood agar to look for contamination. The isolates from LJ culture and MGIT 960 tubes were subjected to MPT64 antigen immunochromatographic test (ICT) BD MGIT TBc Identification test (Becton, Dickinson and company, Sparks, MD, USA). Isolates positive for both acid fast bacilli (AFB) and ICT were identified as MTB complex (MTBC). Isolates positive for acid fast bacilli (AFB) and negative by ICT were identified as non-tuberculous mycobacteria (NTM).

Drug susceptibility testing

Isolates confirmed as *M. tuberculosis* were tested against rifampicin, isoniazid, ethambutol and streptomycin by BACTEC MGIT 960 system using SIRE kit (Becton and Dickinson, Mumbai, India) and standard economic variant of 1% proportion method using LJ medium.

Statistical analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS®) for Windows® release 21.0 (SPSS Inc., Chicago, IL, USA). *P* value < 0.05 was considered to be statistically significant.

Ethical statement

Institute ethics committee approval was obtained from our Institute (Approval No. JIP/IEC/SC/2015/12/722).

Results

Of the 2411 pulmonary specimens obtained from the clinically suspected pulmonary TB patients, 190 (7.0%) were found to be mycobacteria positive by MGIT 960 and 153 (5.7%) by LJ culture. Among the total mycobacteria grown by MGIT 960, 169 (88.9%) were identified as MTB complex (MTBC) and 21 (11.1%) as NTM. Of the 153 mycobacteria grown by LJ culture, 138 (90.2%) and 15 (9.8%) were identified as MTB and NTM, respectively (Table 1). The sensitivity of the liquid culture MGIT 960 and solid LJ culture for MTB were 86.7% and 70.8%, respectively (p value 0.001). The sensitivity of the liquid culture MGIT 960 and solid LJ culture for NTM were 84.0% and 60.0%, respectively (p value 0.180). Contamination rate associated with MGIT 960 and LJ culture was 5.2% and 4.7% respectively and was found not to be statistically significant (P value 0.323).

	MGIT (n = 2411)	LJ (n = 2411)
MTB	169 (7.0)	138 (5.7)
NTM	21 (0.9)	15 (0.6)
Negative	2096 (86.9)	2145 (89.0)
Contaminated	125 (5.2)	113 (4.7)

Table 1: Comparison of MGIT and LJ for the recovery of mycobacteria.

The time to detection of MTB was found to be 18.17 ± 8.69 and 32.46 ± 10.46 days by MGIT and LJ culture, respectively (P value < 0.0001). The time to detection of NTM was found to be 18.76 ± 10.67 and 21.00 ± 12.12 days by MGIT and LJ culture, respectively (P value 0.5611) (Table 2).

Among AFB smear positive specimens, the positivity of MTB by MGIT 960 and LJ were 77.6% and 77.6%, respectively (P value 1.000). With regard to AFB smear negative specimens, the positiv-

Organism	MGIT	LJ	P value
MTB	18.17 ± 8.69	32.46 ± 10.46	< 0.0001
NTM	18.76 ± 10.67	21.00 ± 12.12	0.5611

Table 2: Comparison of MGIT and LJ in the estimation of time to detection (TTD) for MTBC and NTM.

ity of MTB by MGIT 960 and LJ were 4.7% and 3.4%, respectively (P value < 0.0001) (Table 3). The agreement between MGIT and LJ was estimated to be 92.6% (Kappa 0.665) (Table 4).

Smear	MGIT				LJ			
	MTB (%)	NTM (%)	NEG (%)	CON (%)	MTB (%)	NTM (%)	NEG (%)	CON (%)
Negative (n = 2335)	110 (4.7)	21 (0.9)	2083 (89.2)	121 (5.2)	79 (3.4)	15 (0.6)	2134 (91.4)	107 (4.6)
Positive (n=76)	59 (77.6)	0	13 (17.1)	4 (5.3)	59 (77.6)	0	11 (14.5)	6 (7.9)

Table 3: Comparison of MGIT and LJ positivity among Smear positive and smear negative specimens.

CON: Contaminated.

		LJ				Total
		MTB	NTM	NEG	CON	
MGIT	MTB	112	0	34	23	169
	NTM	0	11	9	1	21
	NEG	9	2	2053	32	2096
	CON	17	2	49	57	125
Total		138	15	2145	113	2411

Table 4: Agreement between MGIT and LJ for detection of MTB and NTM.

Of the 195 MTB isolates, drug susceptibility testing was performed for 156, the remaining were excluded as they were contaminated. Of the 156, 4 were multidrug resistant (MDR) strains. All the MDR were identified by MGIT 960. The results of MGIT 960 in comparison to LJ proportion method, percentage agreement/ kappa and the sensitivity and the specificity are summarised in table 5. The turnaround time for drug susceptibility testing (DST) by MGIT 960 ranged from 5 to 15 days, while the turnaround time for LJ proportion method was 28 or 42 days.

Discussion and Conclusion

In a developing country like India where the prevalence of both MTB and NTM lung diseases are rising, laboratory diagnosis based on widely used sputum smear microscopy (SSM) cannot distinguish between MTBC and NTM [11]. Although molecular methods such as Line probe assay (LPA) and GeneXpert MTB/RIF are available, they have some limitations. LPA has limited sensitivity in smear negative cases and limited specificity for detection of NTM due to non-specific hybridization. GeneXpert MTB/RIF has limited detection rate in smear negative cases and lacks ability to detect NTM which in turn further needs sputum culture for final detection [12]. MGIT system, a WHO endorsed method was found to have 10% more sensitivity compared to LJ culture [13].

In our study, the sensitivity of the liquid culture MGIT 960 and solid LJ culture for MTB were 86.7% and 70.8%, respectively. The sensitivity of the liquid culture MGIT 960 and solid LJ culture for NTM were 84.0% and 60.0%, respectively. A north Indian study conducted by Mishra., *et al*. in 132 specimens (Pulmonary-81; Extra pulmonary-51) showed increased recovery of mycobacteria by MGIT 960 (94%) compared to LJ (89%) [14]. A comparative study for mycobacterial isolation conducted by Rishi., *et al.* showed 61.83% by MGIT 960 and 44.01% by LJ culture in pulmonary speci-

Antibiotic	MGIT 960 DST	LJ Proportion method		Percentage Agreement (95% CI)	Kappa	Sensitivity (%)	Specificity (%)
		R	S				
Streptomycin	R	7	1	98.72 (97 to 100)	0.87	87.5	99.3
	S	1	147				
Isoniazid	R	9	0	100	1.00	100	100
	S	0	147				
Rifampicin	R	6	0	100	1.00	100	100
	S	0	150				
Ethambutol	R	10	2	98.08 (96 to 100)	0.86	90.9	98.6

Table 5: Comparison of MGIT 960 drug susceptibility testing with LJ proportion method.

mens with overall isolation rate of mycobacteria 46.9% by MGIT 960 compared to 31.8% by solid media [15]. Satti., *et al.* study in Pakistan showed the increased recovery of mycobacteria (MTBC, 43 and NTM, 1) on BACTEC MGIT 960 to be 97.6% while on LJ medium was 83.7% [16]. Similar to our study, the isolation rate of *Mycobacterium* reported in Portugal, Yugoslavia, Spain was 100% and 90.3%, 93.2% and 67.3%, 95.5% and 79.5% by MGIT 960 and LJ, respectively [13]. An alarming report was documented in South Africa where 89% were detected as MTBC by MGIT 960 after 42 days of incubation out of which 42.3% were multidrug resistant and 1.3% were extensively drug resistant [17].

In our study, we observed that the time to detection of MTB with the MGIT960 system (18.17 ± 8.69 days) was significantly shorter than that with the LJ (32.46 ± 10.46 days). Similar to our results a study conducted for detection of tuberculosis reported the average turnaround time for smear positive was 16 and 31 days and for smear negative samples was 20 and 36 days by MGIT 960 and LJ respectively [13]. Similarly a study in tertiary care centre in India for diagnosis of both PTB and extrapulmonary tuberculosis showed the turn-round time to be around 3 - 40 days by MGIT 960 system and 8 - 42 days by LJ [14]. In our study the time to detection of *Mycobacterium* was not found to be statistically significant for NTM detection by MGIT 960 and LJ. In contrary to our study a multicentre study conducted in Italy showed the time for recovery of NTM was 9 days by MGIT 960 and 22.25 days by LJ and a meta-analysis study by Cruciani., *et al.* showed the time to detection for NTM was 16.3 days by MGIT 960 and 24.3 days by LJ medium [18,19].

In our study both in smear negative cases isolation rate of MTB was higher with MGIT 960 compared to LJ. An Ethiopian study showed, 17.4% and 13.6% of smear negative cases to be positive for MTBC using MGIT 960 and LJ culturemethods respectively [13]. Similarly, an increased recovery of MTBC of 12.3% by MGIT 960 and 9.7% by solid culture in sputum negative cases was shown by Salma Smaoui., *et al* [20].

An important part to be discussed in the liquid culture is the contamination rate. Our study reported a slightly increased contamination rate of 5.2% with MGIT 960 compared to 4.7% with LJ, but this difference was not statistically significant. In contrast to our study, Deepali Saini., *et al*. reports showed lower contamination rate with MGIT 960 than LJ [21]. Similar to our study increased contamination rates was obtained with MGIT 960 (13.8%) compared to LJ culture (6.7%) was reported by Idigoras., *et al* [22]. A study in Bangladesh conducted by Mehedi Hasan., *et al.* also showed higher rate of contamination with MGIT 960 (9.5%) than LJ culture (1.3%) [23].

In our study, the sensitivity of streptomycin, isoniazid, rifampicin and ethambutol were 87.5%, 100%, 100% and 90.9%, respectively. The specificity of streptomycin, isoniazid, rifampicin and ethambutol were 99.3%, 100%, 100% and 98.6%, respectively. In a similar study from China, the sensitivity of streptomycin, isoniazid, rifampicin and ethambutol were reported to be 93.3%, 96.9%, 97.4% and 94.6%, respectively and the specificity of streptomycin, isoniazid, rifampicin and ethambutol were 96.9%, 98.2%, 98.4% and 95.5%, respectively [24]. Therefore, the MGIT 960 has an acceptable sensitivity and specificity for drug susceptibility of MTB for the first line drugs.

We conclude that the overall performance of MGIT 960 for detection of both MTB and NTM is better than LJ culture with rapid recovery of MTB in pulmonary specimens. Our study corroborates

that the application of MGIT960 for detecting both MTB and NTM is most effective and can aid in early and appropriate management of patients with mycobacterial infections. MGIT 960 also has an acceptable sensitivity and specificity for drug susceptibility of MTB for the first line drugs.

Key Messages

MGIT960 has better recovery, with shorter time to detection and similar contamination rate in pulmonary specimens compared to LJ method. The drug susceptibility testing performance of MGIT960 against first line drugs was comparable to LJ.

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