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Original Article

Detection of Drug Resistance Pattern of Mycobacterium Tuberculosis by Genexpert MTB/RIF Solid and Liquid Culture in Extrapulmonary Tuberculosis

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Abstract

Background: *Tuberculosis (TB) remains one of the deadliest communicable diseases. Tuberculosis is caused by Mycobacterium tuberculosis and classified as Pulmonary Tuberculosis (PTB) and Extra Pulmonary Tuberculosis (EPTB). EPTB account for 15-20 % cases of tuberculosis. EPTB diagnosis is challenging due to inadequate sample volume, pauci-bacillary nature and unusual clinical presentation. There are number of tests available for the diagnosis of EPTB but conventional microscopy has low sensitivity and although culture is gold standard method, it takes longer time for yielding positive result. On the other side, GeneXpert due to its rapidity and sensitivity help in early diagnosis and management of tuberculosis. Culture of Mycobacterium Tuberculosis Complex (MTBC) is the accepted reference standard for confirmation of tuberculosis infection and is necessary for Drug Susceptibility Testing (DST). Although solid media has been used for over 100 years, liquid culture media is increasingly being introduced in low and middle income countries for rapid detection of MTBC. To compare the efficacy of solid culture method using Lowenstein Jensen (L-J) media with GeneXpert and liquid culture method using Mycobacterium Growth Indicator Tube (MGIT) 960 for detection of Mycobacterium tuberculosis.*

Materials and methods: *In this cross sectional study 118 suspected EPTB samples including CSF, lymphatic aspirate, pleural fluid, ascitic fluid, synovial fluid, pus were collected. Smear microscopy, culture both in solid media and liquid media using MGIT 960 method and GeneXpert machine were done. Moreover antitubercular drug sensitivity test was done by proportion method in L-J media.*

Results: *Out of 118 samples, GeneXpert detetcs 26 (22.03%) where as the liquid MGIT 960 method detects 27 (22.88%) and solid culture detects 26 (22.03%) positive samples for Mycobacterium tuberculosis. 01 (1.8%) case was detected as multidrug resistant TB.*

Conclusion: *GeneXpert have significant role in diagnosing EPTB patient within shorter period of time. But GeneXpert can detect only rifampicin resistance where as DST in L-J media detects other first line drugs sensitivity too. Liquid culture method using MGIT 960 yields more positive result than solid culture, also liquid culture shows rapid identification of Mycobacterium tuberculosis.*

Key words: Extrapulmonary tuberculosis; GeneXpert; MGIT.

INTRODUCTION

Tuberculosis is an infectious disease caused by Acid Fast Bacilli (AFB) which belongs to the Mycobacterium tuberculosis complex. It typically affects the lungs (Pulmonary TB) but can affect other sites as well (Extrapulmonary TB).[1] Extra-Pulmonary Tuberculosis (EPTB) is any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs. 15%-20% of the total TB cases comprise of Extra-pulmonary TB (EPTH).[2]

Despite the availability of effective antituberculosis chemotherapy for over 50 years TB remains a major global health problem. The situation is worsening in many parts of the world, because of the growing prevalence of drug resistance and the association between tuberculosis and the epidemic of Human Immunodeficiency Virus (HIV) infection. MDR TB has reached the highest prevalence ever documented worldwide in Eastern Europe and Central Asia, where more than 20% of new cases are multidrug resistant.

MDR-TB is caused by Mycobacteria that are resistant to at least Isoniazide (INH) and Rifampicin (RIF) which are most effective anti-TB drugs. Extensively Drug Resistant (XDR) TB involves resistance to the two most powerful anti-TB drugs, rifampicin and isoniazide in addition to resistance to any of the fluroquinolones (Such as levofloxacin or moxifloxacin) and to at least one of the three injectable second line drugs (Amikacin, capreomycin or kanamycin)[3].

Tuberculosis, especially the drug resistant one, appears as a major health problem worldwide. Every year, 10 million people fall ill with Tuberculosis (TB). Despite being a preventable and curable disease, 1.5 million people die from TB each year. An estimated 9.9 million people fell ill with TB worldwide in 2020. Mortality rate globally range from 1.6 to 2.2 million lives per year. The situation is further exacerbated with the increasing incidence of drug resistant TB. Globally in 2013, 3.5% of new TB cases and 20.5% of previously treated cases were estimated to have MDR-TB. Bangladesh ranked 6th among the 22 highest TB burdened countries in the world and 9th among the 25 high priority Multi-Drug Resistant (MDR) and Extensively-Drug Resistant (XDR) TB-flourished .

As per National Tuberculosis Control Program (NTP) report 2019, it is estimated that MDR-TB rate in Bangladesh is 1.4% among new cases and 29% among previously treated cases.

In this context rapid laboratory diagnosis and initiation of early treatment of TB has got a pivotal role in management of TB. In TB endemic areas most of the cases of tuberculosis can be diagnosed correctly by AFB microscopy and Lowenstein Jensen (L-J) media culture. But this traditional bacteriological method possesses several disadvantages. They are either slow or their sensitivity is quite low specially with clinical samples that contain small number of organism. This can adversely affect the treatment by either delaying it or by causing inappropriate empiric therapy for TB . In this situation, not only rapid TB case detection but also the early determination of MDR status is important. The major challenge in the diagnosis of EPTB is the frequently atypical clinical presentation simulating other inflammatory and neoplastic conditions, which frequently results in a delay or deprivation of treatment. Therefore, a high index of suspicion is necessary to make an early diagnosis, and quite often, more than one procedure is necessary for the confirmation of the diagnosis.

New approaches for the rapid detection of mycobacterial growth have been developed with the aim to reduce the time needed for diagnosis as well as treatment. So, the present study was carried out for rapid identification and detection of drug resistance pattern of Mycobacterium tuberculosis by conventional methods, GeneXpert and liquid culture on BACTEC 960 MGIT system in extrapulmonary tuberculosis.

MATERIALS AND METHOD

This cross sectional observational study was conducted in Department of Microbiology, BITID (Bangladesh Institute of Tropical & Infectious Diseases) Chattogram from January 2021 to December 2021. Ethical approval was obtained from the Ethical Review Committee of Chittagong Medical College.

A total of 118 patients with suspected extrapulmonary tuberculosis infection was included in this study on the basis of clinical criteria. Lymph node aspirates, CSF, Pleural fluid, Peritoneal fluid, Synovial fluid and Pus was collected as sample. Smear microscopy, culture both in solid and liquid media and GeneXpert were done for identification of Mycobacterium tuberculosis. Antitubercular drug sensitivity test were also done against first line antitubercular drug. Data was collected using a specially designed case record form.

RESULTS

Out of 118 extrapulmonary samples 01 (0.84%) sample was smear positive , 26 (22.03%) samples were GeneXpert positive in which 1 sample was rifampicin resistant, 26 (22.03%) samples had growth on L-J media and 27 (22.88%) samples had growth on MGIT (Table I).

In 118 extapulmonary samples, out of 38 lymph node aspirates, 6 samples were positive and 15 samples were positive for GeneXpert and culture. Out of 18 samples of pleural fluid 04 samples were positive for GeneXpert and L-J media and 3 samples were positive in MGIT. Out of 14 samples of pus 1 sample was positive for GeneXpert, L-J media and MGIT. In 12 samples of ascetic fluid only 1 sample was positive in MGIT and in 9 samples of synovial fluid 1 samples was positive in MGIT. (Fig 1)

Out of 26 culture positive sample 1 sample were found resistant to rifampicin (Figure 2).

Table I Distribution of results as per the different diagnostic test (n=118)

Diagnostic test	Positive n(%)	Negative n(%)	Total
Z-N Stain	01 (0.84%)	117 (99.16%)	118
GeneXpert	26 (22.03%)	92(47.24%)	118
L-J media culture	26 (22.03%)	92 (57.48%)	118
MGIT	27 (22.88%)	91 (77.11%)	118

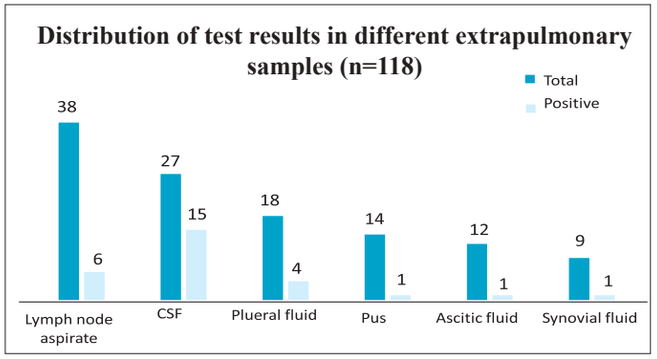

Figure 1 Distribution of test results in different extrapulmonary samples (n=118)

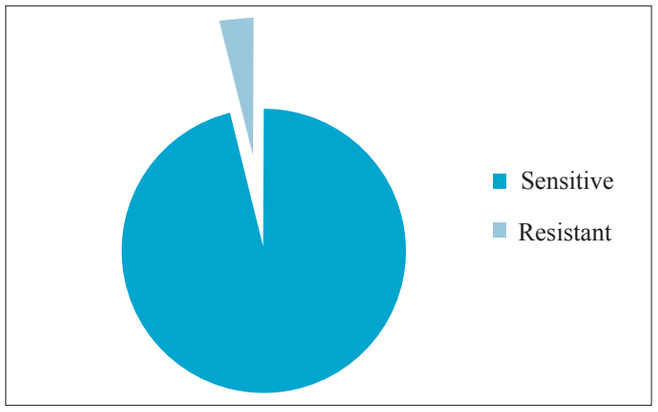

Figure 2 Results of antibiotic sensitivity tests among the culture positive patients (n = 26)

Among 118 extrapulmonary samples 26 (100%) samples are positive for both GeneXpert and LJ media when growth on LJ media taken as a reference standard (Table II).

In TableIII there is a comparison between GeneXpert and Culture on MGIT 960 system, which shows 24 samples are positive for both GeneXpert and MGIT, 2 samples are only positive for GeneXpert, 3 sample was only positive by MGIT 960 system.

Among 118 extra pulmonary samples 24 (92.30%) samples are positive for both LJ media and MGIT, 3 (11.53%) samples are only positive for MGIT and 02(7.69%) samples are only positive by LJ media.(Table IV)

Table II Association between solid culture and Gene Xpert test results among the study subjects (With χ^2 test significance)

Gene test	Xpert-MTB Positive	Solid Culture (LJM) Negative	Total	χ^2 test Significance
Positive	26	0	26	$\chi^2 = 182.590$
				p <0.001
				VHS
Negative	0	92	92	
Total	26	92	118	

- Figures within parentheses indicate percentages.
- VHS = Very Highly Significant (p < 0.001).

Table III Association between liquid culture and Gene Xpert test results among the study subjects (With χ^2 terst significance)

Gene Test Xpert-MTB	Liquid Culture (MGIT) Positive	Negative	Total	χ^2 test Significance
Positive	24	02	26	$\chi^2 = 170.868$
				P = 0.000
				VHS
Negative	03	89	92	
Total	27	91	118	

- Figures within parentheses indicate percentages.
- VHS = Very Highly Significant (p < 0.001).

Table IV Association between solid culture and liquid culture results among the study subjects (With χ^2 terst significance)

Liquid Culture (MGIT)	Solid Culture (LJM) Positive	Negative	Total	χ^2 test Significance
Positive	24	03	27	$\chi^2 = 206.299$
				P = 0.000
				VHS
Negative	02	89	91	
Total	26	92	118	

- Figures within parentheses indicate percentages.
- VHS = Very Highly Significant (p < 0.001).

DISCUSSION

Tuberculosis is a major and global public health problem. The use of less sensitive conventional methods for the diagnosis have contributed to the difficulties in managing patients with extrapulmonary tuberculosis. Main problems begin with clinical specimens containing very few mycobacterium bacilli and their slow growth rate limits their detection by the conventional methods such as acid-fast staining and mycobacterial culture. The early diagnosis of tuberculosis helps in initial treatment and thus preventing the possible transmission of the infection.

Out of 118 extrapulmonary sample, *M. tuberculosis* was isolated in 26 (22.03%) sample when cultured in LJ media and 27 (22.88%) sample when cultured in MGIT 960 system, 26 (22.03%) were detected by GeneXpert out of which 1 sample was Rifampicin resistant and 25 were Rifampicin sensitive. In concordance with present study, there are similar studies that showed increased isolation with MGIT 960 compared to LJ culture. A study in 2020 isolated 42 (28%) *M. tuberculosis* out of 150 samples by MGIT 960 system and 59 (39.03%) by GeneXpert in India.[5] Another study conducted on 66 extrapulmonary samples showed 46.9% isolation rate of mycobacteria by MGIT 960 and 31.8% isolation rate of mycobacteria by LJ culture.[4] Another study in China on 103 extrapulmonary specimens showed the recovery rate of mycobacteria by MGIT 960 systems and LJ medium was 75.73% and 43.72%, respectively.[6]

Among 118 extrapulmonary samples 26 (100%) samples were positive for both GeneXpert and LJ media when growth on LJ media taken as a reference standard.

Comparison between GeneXpert and Culture on MGIT 960 system shows, 24 samples were positive for both GeneXpert and MGIT, 2 samples were only positive for GeneXpert, 3 sample was only positive by MGIT 960 system. Another study found 28% of all extra pulmonary samples tested positive for TB MGIT culture where as 39.33% was tested positive by GeneXpert.[6] In a study by Habous, of 168 non respiratory samples, 52 samples were positive by both culture and Xpert MTB/RIF, 9 samples were detected positive only by culture.[7]

In our study among 118 extra pulmonary samples 24 (92.30%) samples were positive for both LJ media and MGIT, 3 (11.53%) samples were only positive for MGIT and 02(7.69%) samples were only positive by LJ media. In concordance with present study, there are similar studies that showed increased isolation with MGIT 960 compared to LJ culture. Study conducted on 66 extrapulmonary samples showed 46.9% isolation rate of mycobacteria by MGIT 960 and 31.8% isolation rate of mycobacteria by LJ culture.[8,9] Another studies on 103 extrapulmonary specimens showed the recovery rate of mycobacteria by MGIT 960 systems and LJ medium was 75.73% and 43.72%, respectively.[10,11]

With a three specimen batch, the average turnaround time (Including time taken to process specimens and testing time) of the GeneXpert MTB/RIF assay was significantly shorter (2.5 hours) than that of the M960 system (5–42 days). A shorter turnaround time can help TB patients be diagnosed early and treated in time. Therefore, the GeneXpert MTB/RIF assay has an obvious advantage in management of TB.

CONCLUSION

Though culture is considered as a gold standard method for identification of Mycobacterium tuberculosis but it takes several weeks to yield positive outcome as well as for detection of drug resistance. GeneXpert can be a useful diagnostic method for early diagnosis of patients with high clinical suspicion of EPTB. The use of liquid media MGIT-960 is more accurate and rapid for the diagnosis of TB than the conventional solid culture method using L-J media.

RECOMMENDATION

GeneXpert and MGIT 960 is preferred than the conventional method for early diagnosis and initiation of treatment of extrapulmonary tuberculosis. GeneXpert can only detect resistance against rifampicin, culture should be done simultaneously for identification and detection of resistance pattern of other antitubercular drugs.

DISCLOSURE

All the authors declared no competing interest.

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