Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(4):657-660

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Isolation pattern and drug susceptibility scenario in *Mycobacterium tuberculosis* isolates from infected patients of Kashmir Valley, India: A comparative study

Zafar Nowshad[1]*, Javid Khosa[3] and Abhishek Mathur[2]

[1]*Dept of Microbiology, Himalayan University, Arunachal Pradesh, India*
[2]*National Centre of Fungal Taxonomy (NCFT), New Delhi & Institute of Transgene Life Sciences (ITLS), Dehradun, Uttarakhand, India*
[3]*Intermediate Reference Laboratory, Srinagar, Kashmir (J&K), India*

ABSTRACT

The present study was performed in order to compare the isolation and drug sensitivity testing (DST) methods for Mycobacterium tuberculosis culture using solid media (Lowenstein-Jensen/LJ) and liquid media (BACTEC Mycobacterium growth indicator Tube-MGIT 960). This was a cross-sectional survey of adults who visited Intermediate Reference Laboratory, Srinagar (J&K), India with new diagnosis of pulmonary tuberculosis (TB) or failing the first- line TB treatment. Patients were requested to provide two sputum specimens for smear- microscopy and culture on solid and liquid media. Amongst 854 samples, 642 (75.17%) were positive, 211(25%) were found negative and 1(0.1%) were non-tuberculosis mycobacterium (NTM) when isolated through solid/LJ media while 735 (86.06%) were positive, 100 (12%) were negative and 19 (2.22%) were found NTM when isolated through liquid/BACTEC-GIT-960 media. Amongst the two media for isolation of Mycobacterium in random screening procedures, liquid media/BACTEC-MGIT-960 increases diagnosis of TB-positive samples and specifically those with MDR-TB. The choice of culture method should also depend on local availability, cost and test performance characteristics. It was found that, positive cultures of TB were found to be most resistant against streptomycin and most sensitive to ethambutol. The pattern of resistance against drugs in Mycobacterium tuberculosis as per the study follows the order viz. streptomycin>isoniazid>rifampicin>ethambutol. The pattern of sensitivity follows the order viz. ethambutol>rifampicin>isoniazid>streptomycin.

Keywords: Multi-drug resistant tuberculosis (MDR-TB), first-line anti-TB drugs, solid media/LJ, liquid media/BACTEC-MGIT-960, drug sensitivity.

INTRODUCTION

The culture of Mycobacterium tuberculosis complex (MTB) is the accepted reference standard for confirmation of TB Infection and is necessary for drug susceptibility testing (DST). There are several methods for culturing MTB using solid and liquid media. Although solid media has been used for over 100 years, liquid culture media is increasingly being introduced in low and middle-income countries. Solid culture media is cheaper and more widely available, but is labour- intensive, less sensitive and slower than liquid culture [1-3]. Liquid culture systems can be automated, facilitating the processing of large numbers of specimens, but are costly and more prone to contamination. Clinical response to treatment is often less than optimal and WHO estimates that 2.2% and 9.4% of new and re-treatment TB cases have multi-drug resistant TB (MDR-TB). Revised national TB control programme in India has given high priority to improving the identification and treatment of cases with MDR-TB. Previous study

reported the mutational changes in rpoB gene and resistance to rifampicin in *Mycobacterium tuberculosis* strain [4]. The present study was carried out in Intermediate Reference Laboratory, Srinagar (J&K), India in order to compare the isolation rate of MTB from sputum using solid/LJ and liquid/BACTEC-MGIT-960 media and to compare the level of agreement of drug sensitivity testing to first line anti-TB drugs using each type of media. Different studies performed time to time for confirming the most suitable technology for isolation of *Mycobacterium* species [5].

EXPERIMENTAL SECTION

Procedure

Adults greater than 18 years old with sputum smear –positive microscopy were enrolled from April, 2013 to June, 2015. Both newly diagnosed patients and those who have failed first-line treatment (patients continuing to be smear-positive after 5 months of treatment) were prospectively enrolled until 650 new and 204 re-treatment patients were enrolled. Patients enrolled were not receiving treatment at the time of enrolment. Exclusion criteria included patients unable to produce sputum or to provide informed consent to participate in smear negative cases. The sample sizes were calculated to assess the prevalence of drug resistance in new and retreatment patients as described [6]. Specimens considered of poor quality (salivary) or to have a quantity insufficient for both cultures were excluded. Three sputum samples were submitted from each patient for direct Ziel Nielsen smear microscopy. If the patient was smear-positive, the best quality specimen (defined as most mucopurulent) was sent for culture and DST at Intermediate Reference Laboratory Srinagar within seven days of collection. Samples were refrigerated prior to shipment and maintained in cold-chain during shipment with ice packs. At IRL, specimens were decontaminated using Petroff's method (4% w/v NaOH) and cultured on LJ and on an automated BACTEC-MGIT-960 system. Two hundred microlitres of decontaminated sputum were inoculated on each of two glycerol enriched LJ slopes and 500 microlitres in BACTEC-MGIT-960 7H9 bottles. The BACTEC 960 was enriched with supplement (OADC) and antibiotic (PANTA) prior to inoculation. Cultures were incubated at 37^0 C for up to 8 weeks on LJ and 42 days in BACTEC-MGIT-960.LJ cultures were checked weekly and BACTEC-MGIT-960 cultures were monitored continuously through the automated system. Isolates were confirmed as MTB by ZN smear of isolates to observe the serpentine cords typical of MTB, the nitrate reduction test and temperature liability of the catalase test. The time to MTB detection on LJ and BACTEC-MGIT-960 were recorded. MGIT time to detection was defined as the interval between inoculation and the bottle being flagged as positive by the machine. LJ time to detection was defined as the time between inoculation and the culture being considered positive by naked eye reading. Pure AFB cultures were further tested for their sensitivity to streptomycin (STR), isoniazid (INH), rifampicin (RIF) and ethambutol (EMB) using the proportion method in LJ and in MGIT bottles in the BACTEC-MGIT-960 according to the manufacturer's recommendations. Drug concentrations in LJ and BACTEC-MGIT-960 were 8.0 and 1.0 microgram/ml for streptomycin, 0.2 and 0.1 microgram/ml for INH, 40 and 1.0 microgram/ML for Rifampicin and 2 and 1.0 microgram/ML for ethambutol [7-9].

Statistical analysis

All data were analyzed using Epi-info ^{TM} tool. Proportions were compared using chi-square tests and the degree of agreement was compared using the kappa statistic.

RESULTS AND DISCUSSION

A total of 854 sputum samples were cultured on both LJ and BACTEC-MGIT-960 respectively. The mean age range of the patients was 34 (9-85) years, with 315 (60%) being male. The mean (range) time to detection was 11(1-33) and 30 (7-56) days for BACTEC-MGIT-960 and LJ respectively. The results confirmed by the participating centers are shown in **Table 1**. Amongst 854 samples, 642 (75.17%) were positive, 211(25%) were found negative and 1(0.1%) were non-tuberculosis mycobacterium (NTM) when isolated through solid/LJ media. In comparison, total of 735 (86.06%) were positive, 100 (12%) were negative and 19 (2.22%) were found NTM when isolated through liquid/BACTEC-GIT-960 media. The results are shown in **Table 2**. It was found that, positive cultures of TB were found to be most resistant against streptomycin and most sensitive to ethambutol. The pattern of resistance against drugs in *Mycobacterium tuberculosis* as per the study follows the order viz. streptomycin> isoniazid> rifampicin> ethambutol. The pattern of sensitivity follows the order viz. ethambutol>rifampicin>isoniazid>streptomycin. The results of drug sensitivity testing are shown in **Table 3** and **Figure 1**. Tuberculosis drug resistance is increasing in many countries. Most National TB control Programmes are strengthening their capacity for the treatment of MDR-TB but despite these steps many patients are unconfirmed about their infection with TB. The methods by different researchers are not restricted for a single and effective one. Among the aspects that need to be considered are the

sensitivity of the method and its susceptibility to contamination. Services should also be aware of the delay of culture methods which affect clinical management. Liquid media have been reported to detect more cases of first-line drug resistance than LJ media [10-15]. Nowadays different researches are in progress for exploring novel/natural molecules which can be utilized as anti-TB drug and different techniques have been utilized to determine the pathogenecity level of mycobacterium [16, 17].

Table 1: Observation of *Mycobacterium tuberculosis* cultures

	Smear DMC*	Lab smear	Culture Results
Positive	638 (74.7%)	800 (93.7%)	848 (99.3%)
Negative	216 (25.3%)	54 (6.3%)	0
NTM	0	0	1 (0.12%)
Contaminated	0	0	5 (0.58%)
Total	**854**	**854**	**854**

**DMC (designated microscopy centre for Ziehl-Neelsen staining to detected tuberculosis in villages and peripheries)*

Table 2: Culture status confirmation from BACTEC MGIT-960 and LJ media by study site

Status of isolated cultures	*Mycobacterium tuberculosis* isolates quantity and percentage	
	Liquid/BACTEC-MGIT- 960 media	Solid/LJ media
Positive	735 (86.06 %)	642 (75.17 %)
Negative	100 (12%)	211(25%)
NTM	19 (2.22%)	01(0.1%)
Total	**854**	**854**

Table 3: Percent drug sensitivity testing (DST) of *Mycobacterium tuberculosis* cultures in liquid media/BACTEC-MGIT-960

Status of susceptibility of *Mycobacterium tuberculosis* (%)	Streptomycin (S)	Isoniazid (H)	Rifampicin (R)	Ethambutol (ET)
Sensitive	627 (73.41%)	640 (74.94%)	693 (81.14%)	750 (87.82%)
Resistant	217 (25.40%)	204 (23.88%)	151 (17.68%)	94 (11%)
	Total	**854**	**854**	**854**

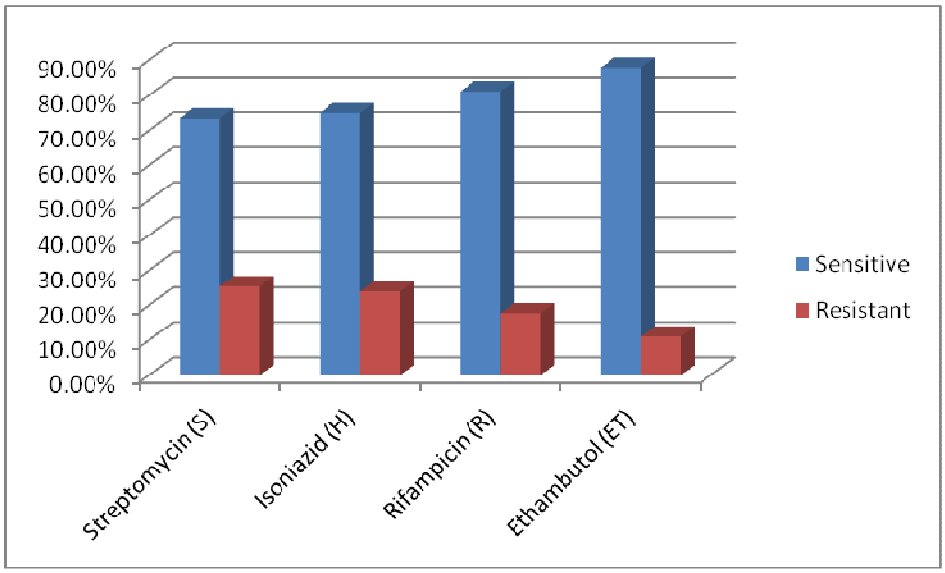

Figure 1: Percent drug sensitivity testing (DST) of *Mycobacterium tuberculosis* cultures in liquid media/BACTEC-MGIT-960

CONCLUSION

The study showed that liquid/BACTEC-MGIT-960 media is much efficient for isolation of *Mycobacterium tuberculosis* (MT) bacterium in comparison to solid/LJ media. Liquid/ BACTEC-MGIT- 960 media is significant in efficient MT isolation with great accuracy and thus is more sensitive than solid/LJ media. The LJ and BACTEC-MGIT-960 culture results were concordant in 450 (85%) samples, with substantial agreement between the methods (kappa= 0.7, standard error=0.046). Thus for random screening of MT isolates liquid media is much more

preferable. Different natural molecules should be investigated and screened in order to have profound effect on Mycobacterium tuberculosis which doesn't show any resistance to the conventional drugs.

REFERENCES

[1] HP Chien, *Int J Tuberc Lung Dis*, **2000**, 4, 90, 866-870.
[2] LQ Srisuwanvilai, *Diagn Microbial infect Dis*, **2008**, 61, 4, 402-407.
[3] L. Lawson, *Trop Med Int Health*, **2011**, 16, 8, 947-980.
[4] Z. Nowshad, A Mathur, KR Kumar, *World J Pharm Pharm. Sci*, **2015**, 4, 7, 985-992.
[5] AK, Asthana, M Madan, *Int J Pharm Bio Sci*, **2015**, 6, 4, 844-847.
[6] F Ardito, *J Clin Microbial*, **2001**, 39, 12, 4440-4444.
[7] L Lawson, *Ann Afr Med*, **2010**, 9, 3, 184-187.
[8] F Bwanga, *Int J Tuberc Lung Dis*, **2010**, 14, 7, 890-895.
[9] L Sangare, *Int J Tuberc Lung Dis*, **2010**, 14, 11, 1424-1429.
[10] EO Idigbe, *J Trop Med Hyg*, **1992**, 95, 3, 186-191.
[11] WHO Policy guidance on TB drug susceptibility testing (DST) of second- line drugs (SLD), **2015**.
[12] IW Fawcett, BJ Watkins, *Tubercle*, **1976**, 57, 1, 71-73.
[13] S Rishi, *Indian J Med Microbial*, **2007**, 25, 4, 383-386.
[14] L Fortoli, J Clin Microbial, **1999**, 37, 11, 3578-3582.
[15] A Sornoskovi, *J Clin Microbial*, **2000**, 38, 6, 2395- 2397.
[16] RS Khare, S Banerjee, K Kundu, *Int J Pharm Bio Sci*, **2011**, 2, 3, 488-500.
[17] M Sabiullah, SL Narayana, J Madhavilatha, MG Devi, VR Kumari, R Rahmathunissa, RB Rao, *Int J Pharm Bio Sci*, **2015**, 6, 3, 141-147.