Evaluation of Microscopic Observation Drug Susceptibility (Mods) Assay for the Early Detection of Drug Resistance in Pulmonary Tuberculosis

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ABSTRACT

Tuberculosis is a major public health problem and drug resistance has worsened it. Early diagnosis and appropriate anti-tubercular regimen is the only solution to this global chaos. But in spite of a diverse array of diagnostic tools, rapid and affordable one to fulfill the current need have not been met. This study, conducted in eastern Nepal evaluated microscopic observation drug susceptibility (MODS) assay for the early detection of Mycobacterium tuberculosis and its drug susceptibility (DST) to Isoniazide and Rifampicin. Seventy-five smear positive sputum specimens were subjected to culture and DST by MODS and conventional method using LJ medium. Though the sensitivity of mycobacterial growth detection was almost similar for both methods, MODS detected growth significantly faster ($p<0.001$), in a median of 10.7 days. Similarly, DST results were also achieved faster by MODS indicating MODS as a reliable method in low resource settings for early detection of drug resistance in pulmonary tuberculosis.

Keywords: Culture, drug susceptibility, Isoniazide, LJ medium, Rifampicin

INTRODUCTION

Tuberculosis (TB) kills more than 2 million people annually; despite being a curable disease.[1] It is the leading cause of death in HIV co-infected persons and adults (15-19yrs) of Africa and Asia.[2,3] Flaring up in condition of poverty, malnutrition and limited access to healthcare, it is still a major public health problem, especially in developing countries. With the emergence of multi drug resistant (MDR) and extensively drug resistant (XDR) TB, the multisectoral burden of this disease is ever increasing, the best solution to which is early diagnosis and initiation of appropriate anti-tubercular regime. MDR-TB has been reported from every region of the world with more than 400,000 cases and more than 100,000 deaths annually.[4] Due to limitation in susceptibility testing it is believed that the true magnitude of MDR problem is larger than currently known. [5]Though, culture remains the gold standard for diagnosis of tuberculosis and mainstay for drug susceptibility test (DST), the time it takes (3-8 weeks) places microscopy as basis for initiation of therapy, which itself can't predict which patient has MDR-TB.[1, 6] This diagnostic dilemma is helping the resistant strain to evolve and propagate in communities where programmes like DOTS are working hard to lessen the global burden. It is an estimation that up to 96% of MDR-TB cases are not being diagnosed and treated appropriately.[7] In response to demand for faster, safer and more accurate diagnostic measures, various approaches ranging from serological tests to molecular methods have come forward but are themselves bounded by various limitations.[8] Microscopic observation drug susceptibility (MODS) assay is based on detection of characteristic cording morphology of Mycobacterium tuberculosis. The growth of Mycobacterium tuberculosis is faster in liquid media and incorporation of anti-tubercular drugs to it permits rapid and direct drug susceptibility test (DST) from specimen. [9] Having high level of sensitivity and specificity, MODS is now in routine use in regional government laboratories in Peru and has gained WHO endorsement as rapid, low cost test for MDR-TB diagnosis.[10, 11] In countries with limited resources settings and high burden of tuberculosis, like Nepal, MODS may be an appropriate diagnostic technique for early detection of MDR-TB.

MATERIALS AND METHODS

This was a laboratory based comparative cross sectional study conducted at Tuberculosis Research laboratory, BPKIHS, Dharan, Nepal from July 2011 to June 2012.

Seventy-five sputum smear positive specimens from new cases who were not on antitubercular treatment were included in the study. Specimens were collected successively, stored in ambient temperature and were processed on weekly basis following standard protocols.

Sputum decontamination[12]

Sputum specimens were decontaminated by the sodium hydroxide–N-acetyl-L-cysteine (NaOH-NALC) method. About 2 ml sputum specimen was taken in a 15 ml centrifuge tube and 2 ml of NaOH-NALC solution was added and the tube was vortexed for 20 seconds, inverted once and left to stand for 15-20 minutes. To neutralize the alkali, phosphate buffer, pH 6.8 was added up to the 14 ml mark in the tube. Inverting the tube 4 times the content was mixed well and the tube was centrifuged at 3000g for 15 minutes. After carefully pouring off the supernatant, the pellet was retained. Almost half of the pellet was removed for culture in LJ medium and remaining was left in the tube for MODS.

Culture and DST by conventional method using LJ medium

The pellet was inoculated into 2 slopes of LJ medium per specimen. An additional p-nitrobenzoic acid (PNB) containing LJ medium was also inoculated and incubated at 37°c. Cultures were examined weekly for up to 8 weeks before discarding as negative.

The isolates were identified as Mycobacterium tuberculosis on the basis of Zeil Nelson staining, slow growth rate, no pigmentation, nitrate reduction test (positive), catalase test (drop method-positive), heat labile catalase test (68°c-negative) and no growth on P-nitrobenzoic acid.

Drug susceptibility test was done by method of proportion on LJ medium (LJ-PM) following standard protocol[13]. Testing was done against two first line antitubercular drugs at the following concentrations: Isoniazide (INH) 0.25µg/ml and Rifampicin (RIF) 40µg/ml.

Simultaneous Culture and DST by MODS[12]

7H9-OADC (for antibiotics) and 7H9-OADC- PANTA (for specimen) were prepared. Using 7H9-OADC, antibiotics working solution of respective concentrations; Isoniazide 4µg/ml and Rifampicin 10µg/ml were prepared according to the alternative method described in the MODS user guideline. Sample suspension was prepared by adding 7H9-OADC-PANTA to the remaining pellet in the centrifuge tube.

A 24 well tissue culture plate was taken for plating the specimens. Each plate had 4 rows, A, B, C and D, and 6 columns, 1 to 6. Each column represented a single specimen. Plating was done in a way that wells of Row A and B were devoid of drugs, wells of row C contained INH and wells of row D contained RIF. On the day of processing, positive control strains (1 susceptible and 1 MDR) were also plated in a separate plate. Plate was closed with its lid and kept inside a zip-lock bag and sealed. Thereafter the plate was incubated at 37°c.

Plate reading was done under inverted microscope from 5th day onwards. Plates were removed from the incubator and subjected to inverted microscopy at 40x magnification with intact zip lock bag. Drug free wells were examined first and if no growth was observed on drug free wells, plates were examined on alternate days thereafter and if no growth was seen by day 21, the plates were discarded as negative. Growth of 2 or more colonies (CFU) , curved or spiral , that later turned irregular and tangled cord like , were taken as positive. When positive results were observed in drug free well, drug containing wells were examined on the same day and results interpreted and validated considering the outcome from positive and negative controls.

RESULTS

Out of 75 specimens, MODS detected positive growth in 59 (78.67%) cases, 11 (14.67%) were contaminated, 3 (4%) were negative in culture and 2 (2.66%) gave indeterminate results. Among those cultured in LJ medium 58 (77.33%) were positive growth, 14 (18.67%) were contaminated and 3 (4%) were negative in culture. Positive growths in LJ medium were subjected to biochemical tests which identified 55 (94.8%) of the isolates to be M.tuberculosis and 3 (5.2%) were Non-Tubercular Mycobacteria (NTM).

Only the result of 59 specimens, excluding the contaminated by any one (7, 9.33%) or either methods (9, 12%) were analyzed head to head. Sensitivity of growth detection by MODS and conventional method was 91.52% and 94.91% respectively. No growth was observed in 3 cases in either methods. Indeterminate result was obtained in 2 cases processed according to MODS. MODS detected mycobacterial growth in an average of 10.7 days (range, 7-19 days), whereas LJ medium took an average of 27.22 days (range, 21-42 days).Regarding drug susceptibility a total of 54 specimens with interpretable growth in either method were evaluated. Six (11.11%) of the isolates were detected as MDR by MODS whereas LJ-PM detected only 5 (9.25%) isolates as MDR. The average time taken for DST was calculated in which it was found that MODS took an average of 10.70 days (range,7-19 days) and LJ-PM took an average of 31.88 days (range, 28-42 days). Likewise, total time taken from processing to result of DST was evaluated in which it was found that MODS took an average of 10.70 days (range, 7-19 days) and LJ-PM took an average of 59.11 days (range, 49-84 days).

Table 1. Head to head analysis of MODS and LJ/ LJ-PM (method of proportion)

Description	MODS		Conventional (L-J/LJ- PM)	
	Number	%	Number	%
Total specimens	59	100	59	100
Growth positive	54	91.53	56	94.92
No growth	3	5.08	3	5.08
Indeterminate	2	3.39	-	-
Average duration for growth in days	10.70		27.22	
Range for growth in days	7-19		21-42	
Sensitivity of detection		91.52		94.91
DST				
Total Tested	54	100	54	100
Sensitive to both drugs	39	72.22	40	74.07
Resistant to any drug	15	27.78	14	25.93
Resistant to RIF	7	12.96	6	11.11
Resistant to RIF only	1	1.85	1	1.85
Resistant to INH	14	25.92	13	24.07
Resistant to INH only	8	14.81	8	14.81
Resistant to both drugs (MDR)	6	11.11	5	9.25
Average duration for DST in days	10.70		31.88	
Range for DST in days	7 - 19		28 - 42	
Average duration (culture + DST) in days	10.70		59.11	
Range for culture + DST in days	7-19		49-84	

DISCUSSION

A large proportion of specimens were from male 52/75(2.26:1) and when age group was considered, 26 (34.66%) of the cases belonged to productive age group (16-30 years) and these were consistent with WHO data for Nepal.[14] A large number of specimens grew contaminant which is much higher for sputum culture by either methods, but in a study by Chaiyasirinroje B et al with 202 clinically diagnosed TB patients in Chiang Rai province, Thailand, the contamination rates of the MODS, and solid media were 15.8% and 10.9% respectively.[15] Indeterminate results reported in MODS were due to absence of growth in one of the drug free well. Evaluation of the two methods head to head for sensitivity of mycobacterial growth detection after excluding the specimens that grew contaminants and taking microscopy as standard, revealed almost similar sensitivity of mycobacterial growth detection by MODS and LJ medium, 91.52% and 94.91% respectively. The higher sensitivity observed in conventional culture (LJ medium) in this study was due to indeterminate results in 2 of the specimens by MODS. If those cases are excluded from evaluation, then the sensitivity of mycobacterial growth detection by both methods will be 94.73%. This is almost similar to the result from Ethiopia by Shiferaw et al in which the sensitivity of mycobacterial growth detection of MODS and LJ medium was 96.9% and 94.3% respectively.[16] The time to culture positivity was compared between MODS and conventional culture (L-J) and it was found that MODS detected mycobacterial growth significantly faster ($P < 0.001$) with an average duration of 10.76 days (range, 7-19 days) in comparison to LJ medium with an average of 27.27 days (range, 21-42 days). Similar result was observed by Michael JS et al in a pilot study in India where the time to culture positivity by MODS and solid reference culture were 10.2 days and 30.2 days respectively.[17] More earlier results were observed by Moore DAJ et al in a MODS-experienced laboratory in Peru in which the average duration to culture positivity by MODS was 7 days and that by LJ medium was 26 days.[9] Earlier detection of mycobacterial growth by MODS seems to depend partly on the use of liquid medium in which Mycobacterium grows earlier and partly on the microscopic observation of the growth, which can detect minute colonies with cording morphology, unable to be seen by unaided eye.

In the test for drug susceptibility against INH and RIF a total of 54 specimens with interpretable growth in either method were evaluated in which similar results were shown by MODS and conventional method. Slight difference in the results between MODS and the LJ proportion method may be due to the qualitative nature of MODS. In MODS, no discrete colonies can be counted, and therefore a resistance proportion cannot be calculated, which is the basic principle underlying LJ-PM. In MODS, growth of ≥2 CFU in drug-containing medium indicates drug resistance, whereas in LJ-PM, growth of more than 1% of that on drug-free medium is interpreted as resistant.

In context of Nepal, WHO in 2009 had estimated only 2.9% of TB among new cases to be MDR.[18] Ministry of health had reported a further less incidence of MDR-TB, 1.3% among new cases.[19] This shows, discordance of result of study conducted in specified center like ours and countrywide data published by governing authorities. But the higher incidence of MDR cases in this study is supported by the estimation of WHO that

96% of MDR-TB cases are not being diagnosed and treated appropriately and further supported by the recent estimation by same organization that only less than 2% of new cases are tested for drug susceptibility globally. [7, 14] This inconsistency may be due to lack of provision of routine DST among new cases and unavailability of such laboratory facilities in most part of our country where only 0.3 DST lab exist per 5 million population.[14]

The time duration for DST was compared between the two methods, in which it was 10.7 days (SD- 2.4 days) for MODS and 31.88 days (SD- 4.64 days) for LJ-PM. When total time from innoculation to result of DST was evaluated MODS was pretty faster again with an average duration of 10.7 days (range, 7-19 days) than culture and DST in LJ medium which took an average of 59.11days (range 49-84 days). Again, the rapid availability of DST result by MODS can be taken as a contribution of the state of culture medium used, method of growth observation and also to simultaneous innoculation of the sample for growth and DST.

In conclusion, this study showed that MODS is significantly faster ($p < 0.001$) than LJ medium in detection of mycobacterial growth from sputum specimen, but almost similar in sensitivity of detection. It also reflects that DST by MODS against INH and RIF is almost similar to that by LJ-PM. So MODS can be used as a tool for early detection of growth and drug susceptibility in cases of pulmonary tuberculosis in low resource settings like ours.

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