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Comparison of drug susceptibility pattern of *Mycobacterium tuberculosis* assayed by MODS (Microscopic-observation drug-susceptibility) with that of PM (proportion method) from clinical isolates of North East India

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ABSTRACT: *About one third of the world's population are infected with tuberculosis and has become a serious global concern of public health. The major problem in controlling tuberculosis is the rapidity and efficacy of detection methods adopted. This study was conducted with the objective to compare the drug susceptibility pattern of Mycobacterium tuberculosis assayed by MODS (Microscopic-observation drug-susceptibility) with that of PM (proportion method) from clinical isolates of North East India. A total of 150 smear positive sputum specimens clinical isolates of M. tuberculosis referred to a clinical lab wa selected. Both the proportion method and MODS were conducted for the collected isolates. DST of MODS were compared with that of PM considering PM as gold standard. MODS detected INH, RIH, STR and EMB resistant isolates at 31% (n = 46/150), 29% (n = 49/150), 19.3& (n = 29/150) and 27.3% (n = 41/150), respectively .Specificity was very high for all the drugs resistance with more than 99% specificity. The accuracy, PPV and NPV of MODS in detection of the four drug resistant isolates was relatively high. DST assay by MODS is relatively simpler. The appreciable performance characteristic in detecting drug resistance including MDR TB may lead to its wider applications in different labs and general hospital for resource limited regions.*

KEYWORDS*: drug susceptibility, MODS, PM, rapid detection, tuberculosis*

I. INTRODUCTION

Tuberculosis arouses public health concern and became a global burden. It is about one third of the population of the world are infected with tuberculosis. AIDS and the increase incidence of multidrug resistant tuberculosis are the major factors that contribute to tuberculosis epidemic. In 2012, 8.6 million people fell ill with TB and 1.3 million died (WHO, 2013).TB occurs in every part of the world. In same year the largest number of new TB cases occurred in Asia, accounting for 60% of new cases globally. However, sub-Saharan Africa carried the greatest proportion of new cases per population with over 255 cases per 100000 populations in 2012 (WHO, 2013).

In 2012, about 80% of reported TB cases occurred in 22 countries. Some countries are experiencing a major decline in cases, while cases are dropping very slowly in others (WHO.2013). India is classified along with the sub-Saharan African countries to be among those with a high burdenfor tuberculosis as well as drug-resistant tuberculosis (WHO, 2012). Though India is the second-most populous country in the world, India has more new TB cases annually than any othercountry. In 2011, out of the estimated global annual incidence of 9 million TB cases, 2.3 million were estimated to haveoccurred in India (WHO, 2013).The nature and frequency of mutations in the resistant strains vary significantly based on the geographical location (Mokrousov et al, 2002). In this regard, here is very less information available on specific mutational patterns in India (Sharma et al, 2003), let alone on the underdeveloped and isolated region of northeast India. MDR TB strains have been reported mostly from countries where HIV and TB co-infection is endemic which includes India (Prasad, 2005).

The dual challenges to TB and HIV co-infections are particularly pressing in northeast India which has the highest average estimated adult HIV prevalence in India (NACO India, 2012). Undiagnosed and mistreated cases continue to drive the epidemic in India. In 2010, an estimated 2.3 million TB cases occurred, and 360,000 patients died of TB, or about 1,000 deaths per day. Nearly one in six deaths among adults aged 15-49 are due to TB. Nearly 100,000 cases of serious multi-drug-resistant TB (MDR-TB) are estimated to occur in the country annually, and each MDR TB case costs more than 1 lakh to diagnose and treat.(Singh et al.,2014)

The incidence:prevalence ratio in India is about 1:32. In case an efficient tuberculosis programme, targeting a sufficient number of sputum-positive prevalence cases in the community, is run for a sufficiently long period of time, it could bring down the prevalence, till probably the point when incidence and prevalence become equal in the community (NACO India, 2012). With an increase in the drug resistance of the Tubercle bacilli, the control of tuberculosis becomes more difficult.

In the present study comparative analysis has been carried out between conventional proportion method (PM) and Microscopic-observation drug-susceptibility (MODS) assay (New Rapid Susceptibility Test). This study also helps in increasing the understanding of TB occurrence and detection methods in NE India.

II. MATERIAL AND METHODS

2.1 Study settings

The clinical isolates referred to the Babina Diagnostic Centre in Imphal, Manipur were taken up for the present study. In order to strengthen the rapid detection of drug susceptibility of *Mycobacterium tuberculosis* at resources limited and high burden region the study was carried out.

2.2 Sputum specimens

One hundred and fifty sputum samples were collected for this study. Entire handling of the clinical specimens was performed inside a Class II safety cabinet in a BSL2 laboratory in accordance with CDC guidelines. Sputum decontaminations were carried out with the conventional N-acetyl-L-cysteine-NaOH. After centrifugation, the pellet was suspended in 1ml of 85% NaCl. All specimens were processed for acid fast microscopy using Ziel-Neesen technique (Canetti et al, 1963; Kent &Kubica, 1985). Sputum samples showing more than 10 acid fast bacilli (AFB) per microscopic field in the smear were selected for the study.

2.3 Critical concentration of antibiotics in Drug Susceptibility Testing

The critical concentration of antibiotics which is given below Table.1 were maintained as per prescribed norms for indirect DST assay for PM and MODS methods.

Table.1. Critical concentration of antibiotics in Drug Susceptibility Testing

Drugs	Critical Concentration (µg/ml)
Isoniazid (INH)	0.2
Rifampicin (RMP)	40
Streptomycin (STR)	4
Ethambutol (EMB)	2

2.4 Proportion method

2.4.1 Culture preparation: Lowenstein - Jensen Medium is used with fresh egg and glycerol for the culture of *Mycobacterium* spp.

2.4.2 Bacterial suspension for inoculation:

Approximately 1mg moist weight of representative sample of the bacterial mass visualized as 2/3 loopful of 3mm internal diameter is added to 0.2ml of sterile distilled water in a 7ml Bijou bottle containing 10-12 glass beads. This mixture is vortexed for approximately 30 seconds to get uniform suspension The suspension is then made up to approximately 1mg/ml concentration by adding more distill water and then kept on the bench for 15-20min to allow coarser particles to settle down. From this suspension, a ten-fold serial dilution is made by adding 0.2ml to 1.8ml sterile distill water. Each serial dilution suspension was inoculated by one standard loopful on to the drug-free as well as the drug-containing LJ slopes

2.4.3 Interpretation of results:

The results are read for the first time on the 28th day. Colonies are counted only on slopes seeded with an inoculum that has produced exact readable counts or actual counts (up to 100 colonies on the slope). This inoculum may be the same for the control slopes and the drug-containing slopes, or it may be the low inoculum (10^{-6} mg of bacilli) for the control slopes and the high inoculum (10^{-4} mg of bacilli) for the drug-containing slopes. The average number of colonies obtained for the drug-containing slopes indicates the number of resistant bacilli contained in the inoculum. Dividing the second figure by the first gives the proportion of resistant bacilli existing in the strain. Below a certain value – the critical proportion – the strain is classified as sensitive; above that value, it is classified as resistant. The proportions are reported as percentages. If, according to the criteria indicated below, the result of the reading made on the 28th day is “resistant”, no further reading of the test for that drug is required and the strain is classified as resistant. If the result at the 28th day is “sensitive”, a second reading is made on the 42nd day and this provides the definitive result. Any strain with 1% (the critical proportion) of bacilli resistant to any of the four drugs – rifampicin, isoniazid, ethambutol, and streptomycin is classified as resistant to that drug. For calculating the proportion of resistant bacilli, the highest counts obtained on the drug-free and on the drug-containing medium should be taken, regardless of whether both counts are obtained on the 28th day, both on the 42nd day, or one on the 28th day and the other on the 42nd.

2.5 Microscopic observation drug susceptibility assay (MODS)

The procedure was carried out as described by Moore et al (2006). MODS is conducted using Middlebrook 7H9 media. Culture preparation was done by dissolving 5.9g of 7H9 medium powder in 900 ml of sterile dw (distilled water) containing 3.1 ml of glycerol and 1.25g of casitone along with PANTA (polymyxin, amphotericin, nalidixic acid, trimethoprim, azlocillin)-to minimize contamination of MODS culture by oral flora micro-organisms not killed during decontamination process. Finally, 100µl INH 4µg/ml (Sigma) or 100µl RIH 10 µg/ml (Sigma) was added to the INH-containing well and RIH-containing well, respectively. The final concentrations of OADC (oleic acid dextrose catalase) and PANTA in each well were 10% and 20 µl/ml,respectively. The drug concentrations in each well were maintained as per given in Table.1 and incubated at 37°Cfor 48 hr to verify sterility (lack of turbidity).

2.5.1 Bacterial suspension for inoculation:

Mix 10 ml sterile dw and 40 µl of 10% sterile Tween 80 in a sterile tube (final Tween 80 concentration = 0.04%). Using a sterile loop, harvest several colonies of *Mycobacteria* and place in a sterile tube containing 100 µl water-Tween 80 solution and sterile glass beads. Cap the tube tightly and vortex for 2-3 min (till there are no visible clumps). Let it stand for 5 min and the open tube and add 3 ml of water-Tween 80. Cap tightly and vortex again for 20s (till suspension has uniform turbidity). Let it stand for 30 min. Transfer the supernatant to another sterile tube using a pipette. Adjust turbidity to McFarland Scale 1 (approximately 3 × 10 CFU/ml) with 0.04% water-Tween 80 solution.

2.5.2 Plate setting for MODS:

A 24 well plate is taken and marked for controls and drugs-containing wells. 900ul of the culture media is dispensed on to the wells and the required drug concentrations are added for drug containing well. The liquid inoculums is then added to make a final volume of 1ml

The plates are then closed with its lids and sealed with paraffin or with zip lock bags and incubated at 37ºC (Sarman et.al; 2012).

2.5.3 Interpretation of results:

The culture is determined to be susceptible when no growth is observed in the both the wells. If one well in either the drug wells has ≥ 2cfu growth while the other well has no growth or is intermediate (≤1cfu is termed as intermediate), then the culture is termed as monoresistant to the drug with growth. If both the drug containing well of the culture has growth ≥ 2cfu then it is considered resistant to both the drugs and is confirmed to be MDR. Fungal or bacterial growth indicates contamination. (Kent,et al 1985; Caviedes et al 2002) .

Table.2.Overall MODS culture interpretation.

Interpretation	
Combined well findings (A&B)	**Overall culture interpretation**
Both wells positive	Positive
Both wells negative	negative
Either well indeterminate	Indeterminate
One well positive, other well negative	Indeterminate
One well positive, other well indeterminate	Indeterminate
Either well contaminated	Contaminated

Statistical methods were performed using SPSS 17.0. Results were considered significant at P value less than 0.5

III. RESULTS

Table 3 below shows the result of DST to the four drugs for PM tested on 150 isolates. Of the total sample, isolates showing resistance to RIH, INH, STR and EMB were 28%(n = 42/150), 31.3%(n = 47/150), 21%(n = 31/150) and 28%(n = 47/150) respectively by PM. Out of the 150 isolates, 26 were detected as MDR due to resistance to both INH and RIH while 16 were monoresistant to RIH and and 21 were monoresistant to INH Table 4). The results of the PM are taken as gold standard for comparison to that of MODS.

Table 3.Drug susceptibility pattern by proportion method

NAME OF DRUGS	RESISTANT	SENSITIVE
RIFAMPICIN	42	108
ISONIAZID	47	103
STREPTOMYCIN	31	119
ETHAMBUTOL	42	108

Table 4.MDR detection by proportion method

	Number of strains (n = 150)
Susceptible	87
RIH mono resistant	16
INH mono resistant	21
MDR	26

Table 5. Drug susceptibility pattern by MODS method

DRUGS	Conventional PM	MODS RESISTANT	MODS SENSITIVE
RIH	RESISTANT	42	0
	SENSITIVE	1	107
INH	RESISTANT	46	1
	SENSITIVE	0	103
STR	RESISTANT	29	2
	SENSITIVE	0	119
EMB	RESISTANT	41	1
	SENSITIVE	0	108

Table.6. Performance of MODS in DST compared with proportion method

Parameters	RIH	INH	STR	EMB
Sensitivity	100(99 – 100)	97.9(95.6 – 100)	93.5(89.6 – 97.5)	97.6(95.2 – 100)
Specificity	99.1(97.5 – 100)	100(99 – 100)	100(99 – 100)	100(99 – 100)
Accuracy	99.3(98 – 100)	99.3(98 – 100)	98.7(96.8 – 100)	99.3(98 – 100)
PPV	97.8 (95.1 – 100)	100(99 – 100)	100(99 – 100)	100(99 – 100)
NPV	100(99 – 100)	99.0(97.5 – 100)	98.4(96.3 – 100)	99.1(97.6 – 100)

Definition of abbreviations: NPV = negative predictive value; PPV = positive predictive value. Values are percentages with 95% confidence interval in parentheses.

Direct drug susceptibility testing results on MODS were compared with indirect DST on LJ as the gold standard for 150 samples. MODS detected INH, RIH, STR and EMB resistant isolates at 31% (n = 46/150), 29% (n = 49/150), 19.3& (n = 29/150) and 27.3% (n = 41/150), respectively shown in Table 3. A comparison of the sensitivity of the four drugs in MODS shows a relatively low percentage in STR (93.5%) with high value in RIH (100%). Specificity was very high for all the drugs resistance with more than 99% specificity. The accuracy, PPV and NPV of MODS in detection of the four drug resistant isolates given in Table 6.

IV. DISCUSSION

Our data shows that MODS is a sensitive and rapid method for diagnosis of TB and DST pattern. The sensitivities in detection of INH and RIH resistance in our study were lower than those from the study of Moore at el 2000 (72.6% vs 84.6% for INH and 72.7% vs 100% for RIH) although both studies used the same INH concentration (0.4µg/ml) and RIF concentration (1µg/ ml). These concentrations have been recommended in the MODS guidelines from the MODS development team in Peru (Jorge et al; 2009. However, a recent meta-analysis published after completion of this study concluded that the sensitivity of INH-resistance detection was higher with a concentration of 0.1µg/ml without loss of specificity (Minion et al., 2010).The only equipment needed to perform the MODS assay are an inverted microscope, tissue culture plate and consumables, biological safety cabinet and incubator. The technical competence required is aseptic technique and microscopy skills. A commercial MODS plate (TB MODS kit™) has been developed by Hardy Diagnostics, USA in collaboration with PATH and is under evaluation.

V. CONCLUSION

In conclusion MODS is appropriate for screening for DST pattern in high burden countries where such tests are urgently needed. MODS meets many criteria for an DST diagnostic test applicable for high-burden settings; it is rapid, low cost and accurate and can be performed without the need for biological safety level 3 laboratories (if the plate is not opened after inoculation). Therefore, MODS is an alternative method for rapid DST screening inthese settings. Recently, wide application of MODS in resource-constrained settings has been endorsed by WHO [2011]. However, an international standard operating procedure and a quality assurance system accredited by WHO should be developed to standardize and maintain accuracy.

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REFERENCES

[1] Canetti G, Froman S, Grosset J, Haudway P, Langerova M, Mahler H T, Meissner G, Mitchison D A and Sula L. Mycobacteris: Laboratory methods for testing drug sensitivity and resistance. *Bull. World Health Organ.* 1963; 29: 565 – 578.

[2] Caviedes L, Deelgado J and Gilman R H. Tetrezoliummicroplate assay as rapid and inexpensive clorimetric method for determination of antibiotic susceptibility of *Mycobacterium tuberculosis. J. Clin. Microbiol.* 2002; 20(40:5):1873 – 1874.

[3] Jorge Coronel MR, Luz Caviedes, David Moore: MODS - A USER GUIDE -Microscopic Observation drug Susceptibility Assay. v12. 114082008. Available at http://www.modsperu.org/MODS userguide.pdf.Accessed Jan 13rd 2009, vol. v12.114082008. Lima - Peru; 2008

[4] Kent, P.T., Kubica, G.P. Public Health Mycobacteriology. A Guide for the Level III Laboratory. US Department of Health and Human Services, *Centers for Disease Control and Prevention, Atlanta*. 1985.

[5] Leimapokpam Shivadutta Singh, Pranab Beheri Mazumder and Gauri Dutt Sharma. Analysis of mutational pattern in multidrug resistant tuberculosis (MDR TB) in a geographically isolated north eastern region of India. *IOSR Journal of Pharmacy and Biological Sciences.*2014;9:4-10.

[6] Microscopic Observation drug Susceptibility Assay. v12.114082008., Available at http://www.modsperu.org/MODS_user_guide.pdf. Accessed Jan 13 rd 2009, vol. v12.114082008. Lima - Peru; 2008.

[7] Minion J, Leung E, Menzies D, Pai M: Microscopic-observation drug susceptibility and thin layer agar assays for the detection of drug resistant tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis* 2010, 10(10):688-698.

[8] Mokrousov I, Narvskaya O, Otten T, Limenschenko E, Steklova L, Vyshnevskiy B. High prevalence of katG Ser315Thr substitution among isoniazid-resistant Mycobacterium tuberculosis clinical isolates from Northwestern Russia, 1996-2001. *Antimicrob Agents Chemother* 2002; 46:1417–1424.

[9] Moore DA, Evans CA, Gilman RH, Caviedes L, Coronel J, Vivar A, et al.Microscopic-observation drug-susceptibility assay for the diagnosis of TB. *N Engl J Med.* 2006 Oct 12; 355(15):1539-50.

[10] National AIDS Control Organisation (NACO), Dept. of AIDS control, Ministry of Health and Family Welfare. *Annual Report* 2011-2012.

[11] Prasad R. MDR-TB: current status. *Indian. J. Tub*.2005; 52:121 – 131.

[12] Sarman Singh, Parveen Kumar, Shreya Sharma, Francis Mumbowa, AnandiMartin,and Nicolas Durie. Rapid Identification and Drug Susceptibility Testing of Mycobacterium tuberculosis: Standard Operating Procedure for Non-Commercial Assays: Part 1: Microscopic Observation Drug Susceptibility Assay v2.4.12.J *Lab Physicians*.2012.4 (2): 101–111.

[13] Sharma M, Sethi S, Mishra B, Sengupta C and Sharma S K. Rapid detection ofmutations in *rpo*B gene of rifampicin resistant *Mycobacterium tuberculosis* strains by line probe assay. *Indian J. Med. Res.* 2003; 117: 76 – 80.

[14] World health Organisation. Global tuberculosis control report. Geneva, Switzerland: WHO, 2011.

[15] World health Organisation. Global tuberculosis control report. Geneva, Switzerland: WHO, 2012.

[16] World health Organisation. Global tuberculosis control report. Geneva, Switzerland: WHO, 2013.