Vaidya et al., 2015

Volume 1 Issue 1, pp.72-89

Year of Publication: 2015

DOI- https://dx.doi.org/10.20319/lijshls.2015.s11.7289

This paper can be cited as: Vaidya, S., Sharma, J., Muley, S., Koppikar, G., Kulkarni, M., & Chowdhary, A. (2015). Comparison of Conventional Method, Radiometric Method and Method Using Oxidation-Reduction Dye for Detection of Multi Drug Resistant Tuberculosis. LIFE: International Journal of Health and Life-Sciences, 1(1), 72-89.

COMPARISON OF CONVENTIONAL METHOD, RADIOMETRIC METHOD AND METHOD USING OXIDATION-REDUCTION DYE FOR DETECTION OF MULTI DRUG RESISTANT TUBERCULOSIS

Shashikant Vaidya
Department of Clinical Pathology, Haffkine Institute for Training, Research and Testing, Mumbai, India
shashikantvaidya@hotmail.com

Jaishankar Sharma
Department of Clinical Pathology, Haffkine Institute for Training, Research and Testing, Mumbai, India
jaishankarsharma199@gmail.com

Shreyasi Muley
Haffkine Bio-pharmaceutical Corporation Ltd. Mumbai, India
Shreyasi.mulye@gmail.com

Geeta Koppikar
Breach Candy Hospital Trust, Mumbai, India
info@breachcandyhospital.org

Mohan Kulkarni
T.N. Medical College and B.Y.L. Nair Charitable Hospital, Mumbai, India

Abhay Chowdhary
Grant Medical College and Sir J.J. Group of Hospitals, Mumbai, India
abhaychowdhary@yahoo.com

Abstract

Today Tuberculosis (TB) has become the most important communicable disease in the world. The emergence of multidrug resistant (MDR) TB has become the main threat to TB treatment and control programs. Rapid detection of TB is critical for the effective treatment of patients. Recently, a method using the Oxidation - Reduction dye has been proposed for drug susceptibility testing of Mycobacterium tuberculosis (M. tuberculosis). In the present study, we have compared Drug Susceptibility Testing (DST) by conventional method, Radiometric method like BACTEC460TB (B460TB) System and Oxidation-Reduction Assay such as Microplate Alamar Blue Assay (MABA) for detection of MDR TB. Total 60 clinical isolates of M. tuberculosis were included in the study. The results obtained by MABA for DST of M. tuberculosis were compared with those obtained by B460TB system and Conventional method. DST results obtained by MABA showed good agreement with results obtained by B460TBsystem and Conventional method for Isoniazid and Rifampicin. In our study specificity and sensitivity for susceptible and resistant strain was found to be 98% and 100% for Rifampicin respectively and 100% and 95% for Isoniazid respectively for MABA.MABA appears to be a reliable method for the rapid and simultaneous detection of MDR-TB and DST of M. tuberculosis. It is simple and inexpensive method for DST of M. Tuberculosis with minimal biohazard risk.

Keywords

Drug Susceptibility Testing, Mycobacterium Tuberculosis, MABA

1. Introduction

Tuberculosis (TB), an old, highly infectious disease, declared a global health emergency by the World Health Organization (WHO) in 1993, is still the second leading killer in the world, with an approximate 2 billion people being latently infected. These latently infected individuals with *Mycobacterium tuberculosis* (*M. tuberculosis)* represent one third of the world's population. WHO estimates that there were approximately 9.0 million new cases and 1.5 million cases of mortality in 2013 and 360,000 of whom were positive for HIV (WHO., 2014). Prevalence of TB in India is fairly high. About 40 % of population is infected and from this pool, cases with clinically active disease continue to develop all the time (Cornwall, 1997)**.**

The incidence of TB is a function of the extent of infection in the community. This renders the provision of permanent diagnosis and treatment facilities as an absolute necessity (Singh, 2004)**.** While prime need is to ensure, by good management and supervision, that resistance does not occur in the first place, surveillance of drug resistance is essential to determine the current scale and nature of drug resistance problem, as well as to define the current solutions. Detection of MDR TB strain would not only eliminate non-essential use of antibiotics, but would also help in selection of the most effective drug regimen and guide therapy in chronic cases (Nunn & Felten, 1994)**.**

In the present study, we have compared conventional method of Drug Susceptibility Testing (DST) that is Résistance Ratio (RR) method and Absolute Concentration (AC) method, radiometric method such as BACTEC460TB (B460TB) System and Oxidation-Reduction Assay such as Microstate Alomar Blue Assay (MABA) for detection of MDR TB.

2. Materials and Methods

Total 60 Clinical isolates of *M. tuberculosis* were collected from Department of Microbiology of P.D. Honduras Hospital and Medical Research centre, Mumbai. All the strains were grown in Sterile Lowenstein Jensen Medium (LJM) slants (Hi Media Laboratories Pvt. Ltd India) with 2% glycerol. All clinical isolates were defined as *M. tuberculosis* according to their growth rates, pigmentation properties of colonies, and susceptibility to para-nitrobenzoic acid, semi quantitative catalase test, nitrate reduction test and niacin accumulation tests. (Vestal, 1975)

DST of all these isolates was carried out by conventional method, B460TB system and MABA (Cannetti, 1963; Franzblau, 1998; Siddiqui, 1981).

DST of isolates was carried out by RR method for drug namely Isoniazid (INH) (Lupin Pharmaceuticals, India)**.** While Rifampicin (RF) (Lupin Pharmaceuticals India) was tested using A.C. Method (Cannetti, 1963).Standard strain of *M. tuberculosis* H37Rv was also tested against all these drugs. Following concentrations (µg /ml) were used for the DST by RR method for INH: 0.05, 0.1, 0.2, 1.0 and 5.0 µg /ml (0.025, 0.05, 0.1 and 0.2µg /ml).Figures in the bracket indicate the concentrations used against *M. tuberculosis* H37Rvfor the DST. Following concentrations (µg /ml) were used for the drug susceptibility testing by A.C. method for RF**:** 1, 2, 4, 8, 16, 32, 40, 64, 128 and 256 µg/ml.

All clinical isolates of *M. tuberculosis* tested by conventional method for DST was tested

by B460TB system by modified Proportion Method (Siddiqui et al, 1981).These clinical isolates of *M. tuberculosis* tested by conventional method and B460TB system were also tested by MABA (Franzblau, 1998). Sterile Dubos broth with glucose and albumin supplement with 0.05% Tween 80 (Hi Media Laboratories Pvt. Ltd, India) was used as growth medium in MABA. Standard strain of *M. tuberculosis* H37Rv was also tested against all the drugs. Following drug concentration (µg /ml) were used for the drug susceptibility testing by MABA.

- INH: 0.012, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 µg/ml
- RF: 0.12, 0.25, 0.5, 1, 2, 4, 8, 16 and 32 µg/ml

Serial two fold dilutions of individual drug were prepared in sterile Dubos broth with glucose and albumin supplement after dissolving it in suitable diluents. 200 µl of sterile water for injection (Haffkine Bio-Pharmaceuticals Co. Ltd, India) was added to all outer perimeter walls of sterile 96 well, micro titreplates, flat bottom with lid (NuncMicroplates co., Denmark). Drug solution of lowest concentration was vortexed and was transferred to sterile troughs.(Lab systems co., UK) By using multichannel pipette,(Lab systems Co., UK) 100µl of drug solution (2X) was added in wells.

In similar way all drug concentrations were added in each column in ascending way. A suspension of test *M. tuberculosis* strain was made in sterile saline to match McFarland number 1 turbidity. Suspension was further diluted to 1:2 in Dubos broth with glucose and albumin supplement. 100µl of test culture inoculums was added to all the wells. Media Control and Positive control were also kept. The plates were sealed with parafilm and incubated at 37^0c for 6 days. On 6^{th} day, 50µl of freshly prepared 1:1 reagent mixture of 10x Alamar Blue solution (Accumed International, Westlake, OH, USA)and 10% Tween 80 solution was added in all the wells of Micro tithe plate. The micro title plates were resealed with parafilm and were incubated for an additional 24 hours at 37^o C and the colour of all wells were recorded. A blue cooler in the well was interpreted as no growth and pink cooler was scored as growth. The Minimum Inhibitory Concentration (MIC) was defined as the lowest drug concentration, which prevented colour change from blue to pink.

3. Results

Table 1: *Analysis of susceptible (S) and resistant (R) strains of M. tuberculosisbyB460TB system and MABA (n=60)*

BACTEC Results					
		INH		**RF**	
MABA Results		**S**	**R**	**S**	**R**
	S	20	1	19	0
	R	0	39	1	40

Total 60 isolates of *M. tuberculosis* were tested by B460TB system and MABA for INH and RF. Total 20 strains were sensitive and 39 were resistant for INH by both the methods. While 1 strain was resistant by B460 TB system and sensitive by MABA for INH. Total 19 strains were sensitive and 40 were resistant for RF by both the methods. While 1 strain was sensitive by B460 TB system and resistant by MABA for RF. Chi-square test was applied to compare the association of B460TB system and MABA for detection of susceptible and resistant strains of *M. tuberculosis* to INH and RF. The methods exhibited significant association for the both the drugs (p-value<0.0001). [Table 1]

Table 2: *Analysis B 460TB system results and MABA results for susceptible and resistant strains of M. tuberculosis (n=60)*

Antitubecular compounds tested	**B460TB system results**		**MABA Results**	
	Susceptible strains	Resistant strains	Susceptible strains	Resistant strains
INH	20	40	21	39
RF	20	40	19	41

Total 60 isolates of *M. tuberculosis* were tested by B460TB system and MABA for INH and RF. Total 20 strains were sensitive and 40 strains were resistant for INH and 20 strains were sensitive and 40 strains were resistant for RF by B460TB system. While 21 strains were sensitive

and 39 strains were resistant for INH and 19 strains were sensitive and 41 strains were resistant for RF. Chi-square test was applied to compare the association of B460TB system and MABA for detection of susceptible and resistant strains of *M. tuberculosis to* INH and RF. The methods exhibited significant association for the both the drugs (p-value<0.0001) [Table 2]

Table 3: *Number of days required for reporting drug susceptibility test results by B460TB system and MABA (n=60)*

Sr. No.	**Number of days**	**MABA Method**	**MABA Method**	**B460TB System**	**B460TB System**
		No. of strains of *M. tuberculosis* evaluated	Cumulative %	No. of strains of *M. tuberculosis* evaluated	Cumulative %
1	1 – 3*	0	0	4	7.3
2	4 – 6*	28	46.67	11	25.66
3	7 – 9	32	100	16	52.33
4	10 – 12	Nil	NIL	15	77.33
5	13 – 15$	Nil	NIL	10	94
6	16 – 18$	Nil	NIL	03	99
7	19 – 21$	Nil	Nil	01	100
Total		60	100	60	100
Average time for Reporting		7.6 days		10.45 days	

(*, $= Rows pooled to apply Chi-square test)

Average time for reporting for MABA was 7.6 days, while for B460TB system was 10.45 days. Applying Chi-square test, the difference between reporting times was significant for both the methods. (p-value<0.0001) [Table 3]

Table 4: *Analysis of sensitivity, specificity and predictive value for susceptible and resistant strains of M. tuberculosis for B 460TB system and MABA (n=60)*

Antitubercular compounds tested	Sensitivity (%)	Specificity (%)	Predictive value for susceptible strains (%)	Predictive value for resistant strains(%)
INH	98	100	95	100
RF	100	95	100	98

MABA indicated high values for the sensitivity and specificity for drug susceptibility testing compared to B460TB system. Sensitivity values were 98% for INH and 100% for RF. While specificity values were 100 % for INH and 95% for RF. Predictive value for susceptible strains for INH was 95 % and for RF 100% while Predictive value for Resistant strains, for INH was 100 % and for RF 98%. [Table 4]

Table 5: *Analysis of susceptible (S) and resistant (R) strains of M. tuberculosis by conventional method and MABA (n=60)*

Conventional method results					
		INH		**RF**	
MABA Results		**S**	**R**	**S**	**R**
	S	20	1	19	0
	R	0	39	1	40

Total 60 isolates of *M. tuberculosis* were tested by MABA and conventional method for INH and RF. Total 20 strains were sensitive and 39 were resistant for INH by both the methods. While 1 strain was resistant by conventional method and sensitive by MABA for Ionized. Total 19 strains were sensitive and 40 were resistant for RF by both the methods. While 1 strain was sensitive by conventional method and resistant by MABA for RF. Chi-square test was applied to compare the association of Conventional test and MABA for detection of susceptible and resistant strains of *M. tuberculosis* to INH and RF. The methods exhibited significant association for the both the drugs (p- value<0.0001). [Table 5]

Table 6: *Analysis of conventional method results and MABA results for susceptible and resistant strains of M. tuberculosis (n=60)*

Antitubecular compounds tested	**Conventional method results**		**MABA results**	
	Susceptible strains (%)	Resistant strains (%)	Susceptible strains (%)	Resistant strains (%)
INH	20	40	21	39
RF	20	40	19	41

Total 60 isolates of *M. tuberculosis* were tested by Conventional method and MABA for INH and RF. Total 20 strains were sensitive and 40 strains were resistant for INH and 20 strains were sensitive and 40 strains were resistant for RF by Conventional method. While 21 strains were sensitive and 39 strains were resistant for INH and 19 strains were sensitive and 41strains were resistant for RF by MABA. Chi-square test was applied to compare the association of Conventional method and MABA for detection of susceptible and resistant strains of *M. tuberculosis* to INH and RF. The methods exhibited significant association for the both the drugs (p-value<0.0001) [Table 6]

Table 7: *Number of days required for reporting drug susceptibility test results by conventional method and MABA (n=60)*

No.	**Number of days**	**MABA Method**	**MABA Method**	**Conventional Method**	**Conventional Method**
		No. of strains of *M. tuberculosis* evaluated	Cumulative %	No. of strains of *M. tuberculosis* evaluated	Cumulative %
1	4 – 6*	28	46.67	Nil	Nil
2	7 – 9	32	100	Nil	Nil
3	19 – 21	Nil	Nil	7	11.67
4	22 - 24	Nil	Nil	11	30
5	25 – 27	Nil	Nil	17	58.33
6	28 – 30	Nil	Nil	25	100

Total		96	100	60	100
Average reporting time		7.6 days		27 days	
X^2 value		**Df**	**p-value**	**Difference is-**	
120.00		5	<0.0001	Significant	

(*= Rows pooled to apply Chi-square test)

Average time for reporting for MABA was 7.6 days, while for conventional method was 27 days. Applying Chi-square test, the difference between reporting times was significant for both the methods. (P-value <0.0001) [Table 7]

Table 8: *Analysis of sensitivity, specificity and predictive value for susceptible and resistant strains of M. tuberculosis by conventional method and MABA*

Ant tubercular compounds tested	Sensitivity (%)	Specificity (%)	Predictive value for susceptible strains (%)	Predictive value for resistant strains (%)
INH	100	95	100	98
RF	98	100	95	100

MABA indicated high values for the sensitivity and specificity for drug susceptibility testing compared to conventional method. Sensitivity values were 100% for INH and 98% for RF. While specificity values were 95 % for INH and 100% for RF. Predictive value for susceptible strains for INH was 100 % and for RF 95%, while Predictive value for Resistant strains, for INH was 98 % and for RF 100%. Average time for reporting for MABA was 7.6 days, while for B460TB system was 10.45 days. Applying Chi-square test, the difference between reporting times was significant for both the methods. (p-value <0.0001) [Table 8]

4. Discussion

Major problem with Mycobacteriological services provided by clinical laboratory is the time factor. Traditional methods used for isolation and identification of tubercle bacilli from clinical specimens are time consuming. The very slow growth rate of *M. tuberculosis* is the limiting factor, since replication time appears to be genetically controlled.

It seems unlikely that any new culture medium devised would significantly reduce replication time of this organism. Therefore one has to look for sensitive technique that can detect very small number of organisms.

Introduction of a liquid selective 7H12 broth medium for culture of Mycobacteria using B460TB radiometric system represented a significant step in decreasing the detection time required for isolation of these organisms. (Morgan et al., 1983; Roberts et al., 1983). The B460TB system also decreased the time necessary to differentiate *M. tuberculosis* from other Mycobacteria and provided a more rapid method for susceptibility testing of *M. tuberculosis*. (Roberts et al., 1983).The B460 TB system was the first broth-based system that could provide rapid results and has been in use for many years. (Gomez-Pastrana et al., 2001). The B460 TB system has been widely validated for approximately 20 years and is regarded as the best method in clinical laboratories for reliable and rapid testing of susceptibility of *M. tuberculosis* isolates to front line drugs such as Streptomycin, Ethambutol, INH and RF in accordance with Center for Diseases control and preventions recommendations. (Scarparo et al., 2004).

But, B460TB system has certain demerits. It is a radiometric method using ^{14}C palmitate which produces 14CO2 by Mycobacterium respiration that collects within the vial and the system is semi-automated which requires constant monitoring and is labor intensive (Luquin et al., 1996).

The major constraint of B460TB system is the cost factor. The cost of isolation, susceptibility and identification testing would work out heavy. These are the direct cost estimate and exclude indirect costs. Oxidation – reduction dyes e.g. tetrazoliums have been used to obtain drug susceptibility measurements for bacteria including Mycobacteria (Gomez-Pastrana et al., 2001). A colorimetric method using a dye, Alamar Blue, for determining the MICs of antimicrobial agents for *M. tuberculosis* has been published. This method provides results, which were in agreement with the agar proportion method in a relatively short period of time. In present study, we described a simple and cheap method that can be applied to detect INH and RF resistance in *M.*

tuberculosis strains. Our study evaluated the performance of MABA with 60 clinical isolates of *M. tuberculosis*.

Antimicrobial susceptibility results obtained by visual Alamar blue method showed good agreement with results obtained by RR method for INH and AC method for RF. In our study specificity and sensitivity and predictive values for susceptible strain and resistant strain was found to be100 % 98%, 95% and for INH respectively, while and 95%, 100%, 100% and 98% for RF respectively**.** Interpretive agreement between MABA method and conventional methods occurred among 174 of 180 susceptibility tests. Every test was performed in triplicate.

Three exceptions, isolates classified as resistant to INH by RR method, were sensitive by MABA method. When they were retested by RR method two were put in resistant category but another remained in susceptible category. By using these criteria, there was interpretive agreement between RR method and MABA method of 96.67 % of the strains tested for susceptibility to INH. The one discrepant result was with strains classified as susceptible to RF by absolute concentration method and classified as resistant by MABA method. By using these criteria, there was interpretive agreement between the Absolute Concentration method and MABA for the 98.3 % of the strains tested for susceptibility to RF. There was significant association in detecting resistant and susceptible strains of *M. tuberculosis* by both methods.

Some studies reported a good correlation between the proportion technique and broth method with Alamar blue, which delivered calorimetric MICs for *M. tuberculosis* isolates in 14 days. It detected 58 % of strains of *M. tuberculosis* on 7^{th} day of incubation by MABA, while remaining 28 % strains were available on 10^{th} day and 4 % strains results were available on 14^{th} day. This study reported only one discrepant result. This study reported 98.3% agreement between Alamar Blue and proportion method for INH and RF sensitivity (Yajko et al., 1995).

MABA MIC test results of all the clinical isolates of *M. tuberculosis* were available by the 7^{th} day of incubation in our study. The reporting time required by MABA and conventional methods showed significant difference.

The results of MABA were compared with those obtained with B460TB system. Antimicrobial susceptibility results obtained by visual Alamar blue method showed good agreement with results obtained by B 460 Method for INH and RF.

In our study specificity and sensitivity and predictive values for susceptibility and resistance

was found to be 100%, 98%, 95% and 100% for INH respectively and 95%, 100%, 100% and 98% for RF. Interpretive agreement between MABA method and conventional methods occurred among 177 of 180 susceptibility tests. Tests were performed in triplicate. Two exceptions were that, isolates classified as resistant to INH by B460TB method were sensitive by MABA method. When they were retested one was put in resistant category but another remained in susceptible category. By using these criteria, there was interpretive agreement between B460TB method and MABA method of 98.3% strains tested for susceptibility to INH.

By using these criteria, there was 100% interpretive agreement between the B460TB method and MABA method of the strains tested for susceptibility to RF. There was significant association in detecting resistant and susceptible strains of *M. tuberculosis* by both methods.

The reporting time required by MABA and conventional methods showed significant difference. Our results were comparable to the results obtained in some of the previous studies. (Franzblau, 1998).

In the present study we followed the protocol of testing of earlier works (Franzblau, 1998). We used 96 welled microstate plates with same incubation temperature and similar conditions. But, instead of 7H9GC broth, we used Dubos broth with albumin and glucose supplement with tween 80. Also we used concentrated culture asinoculums. In this study the dilutions of drugs were used in microstate plate by transferring the drug solutions through columns. While in our study we have prepared the drug dilutions in tubes and overtaxing them thoroughly. These dilutions were then poured in troughs and then added individually in respective columns. We reported the results of drug susceptibility results one day before than them that is 7 days. This might be due the addition of heavy inoculums, preparation of drug dilutions in tubes and addition of albumin, glucose supplement along with tween 80.

Earlier authors evaluated microplate based assay which uses Alamar Blue reagent for antimycobacterial drug screening. Overall MICs determined by visual MABA were highly correlated with those determined in B460TB system. They suggested MABA is sensitive, rapid, inexpensive and non-radiometric and offers the potential for screening with or without analytical instrumentation, large numbers of antimicrobial compounds against slow growing mycobacterium**.** (Collins & Franzblau, 1997)

Some researchers described method for detecting MDR *M. tuberculosis* strains by using

reduction of resazurin. They found high sensitivity and specificity with high predictive values for susceptibility and resistance for INH and RF. This plate method is very similar to the Alamar Blue assay. In this method, interpretation of results was very easy and correlation with proportion method was excellent. Since it has also tested in liquid medium, it has not been implemented as direct DST method due to the contamination problems that may rise. Being a non-proprietary product and cheaper than Alamar Blue, it could be easily implemented in low resource settings, it has the added advantage that it does not require uptake by the bacterial cell. One main concern with this type of test is biosafety, it has been shown, however, that the test can be easily adapted to closed tube format, thereby avoiding this problem. (Palomino & Portaels, 1999)

In 2006, one research group developed and optimized colorimetric nitrate reductive based antibiotic susceptibility test. They found specificity and sensitivity of the test for INH 93.75% and 98.75% and for RF 96.10% and 100 % respectively. The mean reporting time was 6.3 days. This method is reliable, rapid and low cost method for the determination of drug susceptibility pattern of *M. tuberculosis*, particularly in resource poor settings. (Poojary et al., 2006)

Excellent agreement between the results obtained by Nitrate Reductase Assay (NRA) and MABA had been demonstrated. Results were available within 8 days for isolates tested. There was complete agreement between results obtained by NRA and MABA for RF. However, agreement between NRA and MABA was 96% for INH. (Kumar, et al., 2005)

NRA was used as an alternative method for detection of resistance to the first line ant tuberculosis drugs namely INH, RF, EMB and SM. The overall agreement between NRA and proportion method was 98.8%. The NRA was easy to perform and represents a useful tool for rapid and accurate determination of drug resistant *M. tuberculosis* strains in low resource countries. (Montoro et al., 2005)

The assay was developed and assessed a rapid method for Pyrazinamide (PZ) resistance detection in *M. tuberculosis* using nicotinamide in calorimetric reassuring assay The REMA nicotinamide assay demonstrated a sensitivity of 100 % and specificity of 98 %. They found the REMA plate using nicotinamide to detect resistance to PZ is simple and rapid method that could be useful in limited resource countries.(Martin et al., 2006)

The calorimetric method was studied using triphenyltetrazolim chloride for detection of MDR strains of *M. tuberculosis*. They found the specificity and sensitivity 100 % and 92 %

respectively for INH and RF. This method was found to be good alternative for drug testing of *M. tuberculosis* isolates. (Mohammadzadeh et al., 2006)

Our study reported sharp breakpoints for INH and RF. The wells were either blue or pink. Similar observations were made in previous studies. (Franzblau, 1998)

In general the repeat tests results were considered to be more accurate as a result of the additional experience obtained by the technician for whom this study represented the first attempt at performing susceptibility studies in a micro plate format. Sealing of the microplate with parafilm should minimize the biohazard potential in the event that plate is mishandled. Contamination was not found to be problem.

MABA is simple to perform and inexpensive giving results after one week of incubation that are comparable to those of DST methods that use liquid media. For RF resistant mutants MICs were higher than 1 µg/ml, allowing easy discrimination of RF resistance. All of these isolates were also resistant to INH. So testing of RF resistance alone with MABA method would have identified all of the MDR TB isolates.

Existing methods for drug susceptibility testing of clinical *M. tuberculosis* isolates are either inexpensive with long turnaround time or rapid but too expensive for all but the most affluent institutions. The MABA offers a superior combination of rapidity and affordability.

Results from our study and other studies (Franzblau, 1998; Yajko et al., 1995) may allow the selection of one or two critical concentrations of each drug for use in differentiating susceptible, partially susceptible and fully resistant strains. This would further reduce the cost of the assay by allowing the drug susceptibility testing of 3 isolates in one single 96 well plate. The minimum major equipment needed to perform MABA is Biosafety cabinet, an autoclave and incubator.

In comparison to, B460 TB system, sensitivity and specificity of MABA was more than 95 % for INH and RF. Predictive value for resistant strains and sensitive strains of *M. tuberculosis* for INH and RF was more than 95% by MABA.

The significant difference in average reporting time between MABA (7.6 days) and B460 TB (10.45 days) method was observed. There was significant association in reporting resistant and susceptible strains by both the methods. While there was no significant difference in number of susceptible and resistant strains of *M. tuberculosis* encountered by both the methods. Sensitivity and specificity of MABA was more than 95% for INH and RF in comparison to conventional methods.

Also predictive value for resistant strains and sensitive strains of *M. tuberculosis* for INH and RF was more than 95% by MABA. The significant difference in average reporting time between MABA (7.6 days) and conventional (27 days) method was observed. There was significant association in reporting resistant and susceptible strains by both the methods. While there is no significant difference in number of susceptible and resistant strains of *M. tuberculosis* encountered by both the methods.

5. Conclusion

Following conclusions can be drawn from these studies .MABA is found to be highly sensitive and specific method in detecting MDR and susceptible strains of *M. tuberculosis.* This method is comparable to existing methods used for detection of MDR strains. Average reporting time required for drug susceptibility results by this method is shorter than other methods. In India, in poor set up this method can be cost effective, useful and reliable alternative for determining drug resistance. As it provides wide range of precise MICs, which can be used to define susceptibility breakpoints for the older drugs in current use as well as to establish breakpoints for newer agents.

6. Acknowledgement

The authors were grateful to late Dr. Ajita Mehta, Head, Department of Microbiology and Dr. Camilla Rodriguez, Consultant Microbiologist and Chairperson, Infection control Committee, P.D. Hinduja Hospital and Medical Research Center, Mumbai, India for their constant support and valuable guidance.

References

Cannetti, G. S., Froman, J., Grosset, P., Hauduroy, M., Langerora, H.T., Mahler, G., Meissner, Mitchison, D.A. and Sula, L. (1963). Mycobacteria: Laboratory methods for testing drug sensitivity and resistance. *Bull W.H.O., 29*, 565-578.

Collins, L., & Franzblau, S. G. (1997). Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium. Antimicrobial agents and chemotherapy, 41(5), 1004-1009.

Cornwall, J. (1997). Tuberculosis: a clinical problem of international importance. The Lancet, 350(9078), 660-661.

Franzblau, S. G., Witzig, R.S., McLaughlin, J.C., Madico, T.P., Hernandez, G., Michelle, A., Degnan, T., Cook, M.B., Quenzer, V.K., Fergusan, R.M. and Gilman, R.H. . (1998). Rapid low–technology MIC determination with clinical Mycobacterium tuberculosis isolates by using microplate alamar blue assay. J Clin Microbiol, 7, 362-366.

Gomez-Pastrana, D., Torronteras, R., Caro, P., Anguita, M. L., López-Barrio, A. M., Andres, A., & Navarro, J. (2001). Comparison of amplicor, in-house polymerase chain reaction, and conventional culture for the diagnosis of tuberculosis in children. *Clinical infectious diseases, 32*(1), 17-22.

Kumar, M., Khan, I., Verma, V., & Qazi, G. (2005). Microplate nitrate reductase assay versus Alamar Blue assay for MIC determination of Mycobacterium tuberculosis. The International Journal of Tuberculosis and Lung Disease, 9(8), 939-941.

Luquin, M., Gamboa, F., Barceló, M. G., Manterola, J., Matas, L., Giménez, M., & Ausina, V. (1996). Comparison of a biphasic non-radiometric system with Lowenstein-Jensen and Bactec-460 system for recovery of mycobacteria from clinical specimens. Tubercle and Lung Disease, 77(5), 449-453.

Martin, A., Takiff, H., Vandamme, P., Swings, J., Palomino, J. C., & Portaels, F. (2006). A new rapid and simple colorimetric method to detect pyrazinamide resistance in Mycobacterium tuberculosis using nicotinamide. Journalof Antimicrobial Chemotherapy, 58(2), 327-331.

Mohammadzadeh, A., Farnia, P., Ghazvini, K., Behdani, M., Rashed, T., & Ghanaat, J. (2006). Rapid and low-cost colorimetric method using 2, 3, 5-triphenyltetrazolium chloride for detection of multidrug-resistant Mycobacterium tuberculosis. *Journal of Medical Microbiology*, 55(12), 1657-1659.

Montoro, E., Lemus, D., Echemendia, M., Martin, A., Portaels, F., & Palomino, J. C. (2005). Comparative evaluation of the nitrate reduction assay, the MTT test, and the resazurin microtitre assay for drug susceptibility testing of clinical isolates of Mycobacterium tuberculosis. Journal of Antimicrobial Chemotherapy, 55(4), 500-505.

Morgan, M., Horstmeier, C., DeYoung, D., & Roberts, G. (1983). Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-negative specimens. Journal of Clinical Microbiology, 18(2), 384-388.

Nunn, P., & Felten, M. (1994). Surveillance of resistance to antituberculosis drugs in developing countries. Tubercle and Lung Disease, 75(3), 163-167.

Palomino, J., & Portaels, F. (1999). Simple procedure for drug susceptibility testing of Mycobacterium tuberculosis using a commercial colorimetic assay. European Journal of Clinical Microbiology and Infectious Diseases, 18(5), 380-383.

Poojary, A., Nataraj, G., Kanade, S., Mehta, P., & Baveja, S. (2006). Rapid antibiotic susceptibility testing of Mycobacterium tuberculosis: Its utility in resource poor settings. Indian Journal of Medical Microbiology, 24(4), 268.

Roberts, G., Goodman, N., Heifets, L., Larsh, H., Lindner, T., McClatchy, J., . . . Wright, P. (1983). Evaluation of the BACTEC radiometric method for recovery of mycobacteria and drug susceptibility testing of Mycobacterium tuberculosis from acid-fast smear-positive specimens. Journal of clinical microbiology, 18(3), 689-696.

Scarparo, C., Ricordi, P., Ruggiero, G., & Piccoli, P. (2004). Evaluation of the fully automated BACTEC MGIT 960 system for testing susceptibility of Mycobacterium tuberculosis to pyrazinamide, streptomycin, isoniazid, rifampin, and ethambutol and comparison with the radiometric BACTEC 460TB method. Journal of Clinical Microbiology, 42(3), 1109- 1114.

Siddiqui, S. H., Libonati, J.P. and Middlebrook, G. (1981). Evaluation of a rapid radiometric method for drug susceptibility testing of Mycobacterium tuberculosis. J Clin Microbiol, 13, 908-912.

Singh, S. (2004). HIV-TB Co-infection: A deadly combination, international symposium on Tuberculosis research.

Vestal, A. L. (1975). Procedures for the isolation and identification of Mycobacteria. Atlanta center for disease control.

WHO. (2014). Global Tuberculosis Report 2014: World Health Organization.

Yajko, D. M., Madej, J. J., Lancaster, M. V., Sanders, C. A., Cawthon, V. L., Gee, B., . . . Hadley, W. K. (1995). Colorimetric method for determining MICs of antimicrobial agents for Mycobacterium tuberculosis. Journal *of clinical microbiology, 33*(9), 2324- 2327.