

Modified slide culture method for drug susceptibility testing for *Mycobacterium tuberculosis*

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Abstract

Introduction: Current methods for drug susceptibility testing of *Mycobacterium tuberculosis* are either costly or slow. As the prevalence of multidrug-resistant strains increases, the need for fast, reliable, and inexpensive methods that can also be applied in settings with scarce resources is obvious.

Aim: To determine the prevalence of multi drug resistant tuberculosis by a simple, rapid and cost effective technique.

Materials and Method: 100 sputum samples from sputum smear positive patients at RNTCP centre were taken and subjected to drug susceptibility testing for all the first line anti tubercular drugs by modified slide culture method. Samples were also subjected to conventional culture on LJ media.

Results: From the 100 smear positive sputum samples, culture of *M. tuberculosis* was obtained by both LJ medium and modified slide culture for all the samples. Out of the 100 samples, 94 were sensitive for all the drugs, 04 were found to be multidrug resistant and 02 resistant only to isoniazid.

Conclusions: The modified slide culture technique was found to be a safe, rapid and cost effective technique and can be used for drug susceptibility testing especially in resource limited settings.

Keywords: *Mycobacterium tuberculosis*, Sputum sample, LJ media, Modified slide culture, Multi drug resistant

Introduction

Tuberculosis (TB) being one of the destructive disease, has been affecting humanity since a very long time. Due to the rise in number of cases of multi drug resistant tuberculosis (MDR-TB), and extensively drug resistant tuberculosis (XDR-TB), management of tuberculosis has become more challenging.⁽¹⁾ So the time has come where the timely diagnosis of drug resistant cases of tuberculosis would be of great help to the clinician in management. The results of conventional modalities of anti-tubercular drug susceptibility testing usually take a minimum of 10 to 12 weeks. Liquid culture methods like BACTEC MGIT 960 take much lesser time but the initial infrastructure requirement and the cost per test are a bit high to afford in peripheral laboratories. Hence, it has become necessary to search for a simple, rapid and more affordable technique for anti-tubercular drug susceptibility testing. In this study we have tried to determine the prevalence of multi drug resistant tuberculosis (MDR-TB) by modified slide culture technique.

Materials and Method

Sputum samples from smear positive patients visiting our RNTCP centre were collected in a sterile wide mouthed container. The collected samples were subjected to decontaminated and concentrated by modified Petroff's method.⁽²⁾ Biosafety class II cabinet was used for procedure.

Culture of *M. tuberculosis* on LJ media:⁽³⁾ The LJ media used for culture of *Mycobacterium tuberculosis*

was prepared in house. Two LJ media slants were inoculated with two loops full of decontaminated and concentrated deposits in a pre-sterilized inoculation hood taking all necessary aseptic precautions. The Date of inoculation was noted. The slopes were kept for incubation at 37°C for a maximum of 8 weeks. In the first week the slopes were checked for any growth on daily basis. From second week onwards, the slopes were checked for any growth or contamination twice a week. In case of *Mycobacterial* growth, the date of first appearance of colony was noted and slopes were further incubated for further growth. In case of any contamination, the slopes were removed. Growth of AFB on LJ Media was confirmed by observing for Acid Fast Bacilli in the smears made from the colonies. Gram's stain was done to rule out bacterial contamination and LCB mount was done to rule out fungal contamination on the positive slants. Identification of *Mycobacterial* isolates was done by Para Nitro Benzoic Acid (PNB) inhibition tests.⁽⁴⁾ *M. tuberculosis* is sensitive to PNB whereas non tuberculous mycobacteria grow in the presence of PNB.

Modified slide culture method:^(5,6)

Glass slides of thin glass were used. The slides were longitudinally split with the help of a glass knife. The sample identity and drug code were mentioned on each slide. Citrated blood obtained from a Blood Bank was used. Care was taken to see that the blood obtained was not more than 28 days old. An equal amount of sterile deionized water was added and stirred till hemolysis was complete. Polymixin B 200 units/ml, carbenicillin 100 mg/L, trimethoprim 10 mg/L and

amphotericin B 10 mg/l were added to the medium to make it selective. The medium was poured into sterile glass bottles. No anti-tuberculosis drug was added to bottle No. 1. To bottles No. 2, 3 and 4, Isoniazid was added to make drug concentrations of 0.1 mg/L, 0.2 mg/L and 0.4 mg/L respectively. To bottles No. 5, 6 and 7, was added Streptomycin to make concentrations of 1.0 mg/L, 2.0 mg/L and 4.0 mg/L respectively. To bottles No. 8, 9 and 10, Rifampicin was added to make concentrations of 0.2 mg/L, 0.4 mg/L and 0.8 mg/L respectively and to bottles No. 11, 12, and 13, Ethambutol to make 0.5 mg/L, 1.0 mg/L and 2.0 mg/L respectively. Ten ml each of drug free and drug containing media were poured aseptically into sterile 28 ml McCartney bottles. The lower 2/3rd of each split slide was then inoculated with processed sputum sample. 16 such slides were made out of each specimen. These slides were then allowed to dry before being placed into drug free and drug containing media. The remaining slides were kept as control. The slide cultures were kept for incubation at 37°C for 1 week. The slides were then removed with a pair of forceps and the excess of blood were removed by dipping the slide in sterile water for 5 minutes. The back of each slide was wiped clean with cotton before being placed in a slide rack, air dried and then placed in oven for 30 minutes at 80°C. These slides were then stained by Z.N. technique and examined under bright field microscope for presence of micro colonies.

The microcolonies were graded as under:

Negative: No acid fast bacilli seen as compared with an unincubated control smear.

1 + Small clumps of upto 4 bacilli.

2+ Large clumps of bacilli but no cord formation.

3 + Micro-colonies with some cord formation.

4+ Large micro-colonies with good cord formation.

For every 10 sputum specimens examined, one control slide culture was also put up using H₃₇R_v strain of *M. tuberculosis*. Specimens showing growth of grade 3 + or 4+ only were considered for analysis of the sensitivity pattern.

Drug concentrations and definition of resistance:

The minimal drug concentrations of various drugs used which prevented a growth of at least 1 grade less than that on drug free media of H₃₇R_v strain were considered as the critical drug concentrations. Resistance was assumed if a specimen showed equivalent or 1 grade less growth on the critical drug concentration as compared with the drug free media, i.e., if drug free media showed a growth of 3 +, a 2 + or more growth on the critical drug concentration of drug media was considered as drug resistant.

Results

From 100 sputum smear positive samples, culture of *M. tuberculosis* was obtained by all the samples on both slants the slants of LJ media and a 4+ growth was obtained on all controls slide and on the slides placed in

drug free medium by modified slide culture method. Out of the isolates, 94 isolates are sensitive for all the drugs, 04 were resistant to rifampicin and isoniazid and hence were classified to multidrug resistant (MDR)⁽⁷⁾ tuberculosis cases and 02 were resistant only to isoniazid and sensitive to other three drugs. 02 isolates were resistant to ethambutol and streptomycin along with rifampicin and isoniazid.

Table 1: Comparison of results by culture on LJ medium versus Modified Slide Culture method

	LJ Medium culture positive	LJ medium culture negative	Total
Modified Slide culture positive	100	00	100
Modified Slide culture negative	00	00	00
Total	100	100	100

Table 2: Drug susceptibility results by Modified slide culture method

Sensitive Or Resistant To Particular Drug	Number of Cases
sensitive to Isoniazid	94
resistant to Isoniazid	06
sensitive to Rifampicin	96
resistant to Rifampicin	04
sensitive to Streptomycin	98
resistant to Streptomycin	02
sensitive to Ethambutol	98
resistant to Ethambutol	02

Discussion

In this study, only new clinically suspected tubercular cases were selected. Any case already on anti-tubercular treatment was excluded from the study. This may be reason all the 100 samples showed growth by both the conventional culture on LJ media as well as by modified slide culture method. The prevalence of MDR-TB was found to be 4% in our study. Gupta et al⁽⁵⁾ in their study, in all 83 out of the total 100 sputum specimens tested showed a growth of 3+ to 4+ on slide culture. These 83 cultures were further analysed for anti-tubercular drug susceptibility: 66 out of them showed bacillary growth sensitive to all the drugs tested. The remaining 17 showed drug resistance to 1 or more of the drugs. More number of studies is still required to establish the efficacy of modified slide culture method for drug susceptibility testing for *M. tuberculosis*.

Conclusion

Though the sample size is small to comment on the overall prevalence of MDR-TB, we found that modified slide culture method could serve as a safe, rapid and cost effective method for drug susceptibility testing of *M. tuberculosis* especially in resource limited settings.

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