Research Article

Direct Nitrate Reductase Assay: Rapid Detection of MDR-TB in Low Resource Settings

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ABSTRACT

Conventional methods based on measurement of growth in culture media containing antibiotics are available for detection of drug resistance in Mycobacterium Tuberculosis requiring several weeks for results. Newer methods like BACTEC are costly. Hence a simple and rapid alternative method of Nitrate Reductase Assay (NRA) was used in this study to detect resistance to Isoniazid (INH) and Rifampicin (RIF). Sputum samples were collected from patients attending DOTS centre at NKPSIMS from July 2012 to May 2013. Smear AFB Positive samples were only included. After decontamination, 112 sputum were inoculated on plain LJ and 3 Middle Brook 7H9 media (One Plain MB with KNO3, one with KNO3 and INH, one with KNO3 and RIF). Nitrate reduction was tested on Days 7, 10, 14 and 18 of incubation. Control strain H37Rv was used as positive control for nitrate reduction. Eleven samples were contaminated. NRA was performed on 101 samples. Fourteen were resistant to INH, whereas 6 were resistant to RIF and INH. Maximum (46) samples were nitrate positive on day 14. Twenty Eight and 22 samples were positive on day 10 and day 18 respectively. Positivity was seen as early as 7th day in only 5 samples. The present study concludes that this test, being easy, rapid, simple and time saving, can be applied directly on sputum positive patients without waiting for the culture. Thus, NRA can be used as rapid detection test for MDR-TB cases in low resource settings.

KEY WORDS: multidrug resistant tuberculosis (MDR-TB); nitrate reductase assay (NRA)

INTRODUCTION:

Tuberculosis remains a major public health problem worldwide. In recent years, the incidence of Tuberculosis has been rising. There is also an emergence of Multidrug Resistant (MDR) tuberculosis worsening the impact of this disease. Current methods of drug susceptibility testing of M tuberculosis are slow or costly. As the prevelence of MDR strains increases, the need for fast, reliable and inexpensive methods suitable for application in low resourse settings is required[1].

Recently alternative rapid methods have been developed. Among those, the Nitrate reductase assay (NRA) on Lowenstein-Jensen (LJ) Medium is simple to perform[2]. This test is based on the ability of M Tuberculosis to reduce nitrate to nitrite which is revealed as a colour change in the culture medium[3]. In a study done by H.Syre Middlbrook 7H9 broth was used to perform Nitrate Reductase test [4]. In some studies the nitrate reductase assay was applied directly to sputum samples.There was significant reduction of the time needed to obtain the results. The results of these studies were concordant with results obtained by the reference method to detect MDR[5,6]. The present study aimed for rapid detection of MDR tuberculosis cases in a rural area based tertiary care hospital.

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MATERIALS AND METHODS:

Sputum samples were collected from patients attending DOTS centre at NKP Salve Institute of Medical Sciences and Research Centre, Nagpur from July 2012 to May 2013. Of all the sputum samples, only Smear AFB Positive samples were included in this study. The sputum samples were processed by modified Petroff's digestion -decontamination method .The supernatant was discarded. Sediment was resuspended in 3 ml of sterile distilled water. This was used to inoculate the culture media which included

Plain LJ (without antibiotic) and Middle Brook Liquid media.

The liquid medium was supplemented with an antimicrobial mixture containing polymyxin B,nalidixic acid, trimethoprim and azlocillin (PANTA), 0.1 ml per vial to prevent the growth of nonmycobacterial contaminants[7].

LJ medium was used to perform proportion method. For Nitrate reductase test, 3 Middle Brook 7H9 liquid media with Potassium Nitrate were used. (First MB with KNO3,Second MB with KNO3 with 0.2ug/ml of INH and third MB with KNO3 with 40ug/ml of RIF). All tubes were incubated at 37^0c. Nitrate reductase assay was based on ability of Mycobacterium tuberculosis to reduce nitrate to nitrite which was evident by colour change in the medium after addition of Griess reagent (1part 50% concentrated hydrochloric acid, 2 parts 0.2% sulfanilamide, and 2 parts 0.1%N-1-naphtylethyl-enediamine dihydrochloride). Nitrate reduction was tested on 7th day of incubation in drug free MB. If the medium turned pink then tubes with INH & RIF were tested. If there was no colour change then the tubes were reincubated and the procedure repeated on days 10,14 and 18. The results were classified as negative (sensitive) if no colour change occurred in the antibiotic containing media and positive (resistant) if there was pink to red colour development.

Proportion method was performed using LJ medium according to the standard procedure with the recommended critical concentration of INH and Rifampicin[5]. Control strain H37Rv was used as positive control for nitrate reduction.

RESULTS:

Out of 112 smear positive samples, 11 were contaminated. Total 101 were tested by NRA . In this study the 5.94% of the strains showed multi drug resistance. Nitrate reductase test gives rapid results as compared to soild media. As evident from Table II, growth was detected by 14th day in maximum strains. By 18th day, all the results were available. Conventional method using solid medium (LJ) takes more than 3 weeks for growth of M Tuberculosis.

Table 1: Percentage of Drug Resistance (Total n=101).

Antibiotic	Resistant
Isoniazide	14(13.86%)
Rifampicin	6 (5.94%)
Rifampicin & INH (MDR)	6 (5.94%)

By Nitrate reductase test resistance to INH & RIF was detected within 18th day. Maximum MDR cases were detected on 14th day. Conventional method requires 21-28 days for primary isolation prior to performing the drug sensitivity test and about 28 to 42 additional days for the final results.

Table 2: Availability of results by Nitrate Reductase Assay.

No. tested	7^{th} day	10^{th} day	14^{th} day	18^{th} day
101	5 (4.95%)	28 (27.72%)	46 (45.54%)	22 (21.78%)

Table 3: Detection of Drug Resistance by Nitrate Reductase Test.

Resistance	7^{th} day	10^{th} day	14^{th} day	18^{th} day
INH	0	1	9	4
RIF	0	0	4	2
MDR	0	0	5	1

DISCUSSION:

Many studies have shown that NRA test gives concordant results with proportion method. Hence in this study, NRA was directly applied to sputum samples. The results of this study indicates that NRA can be used for rapid diagnosis of MDR TB. About 5.94% strains were multi drug resistant. Maximum results were obtained within 14 days. In a study done by Dissou et al 9% results were available on day 10, 50% at day 14, 37% at day 18 and 4% results were obtained at 28th day[3]. Baijayanti Mishra reported that results were available in 14 days for 28.1% strains, in 21 days for 65.6% strains and in 28 days in 6.3% strains. M. Gupta et al observed that results were available in seven days for 61% of the strains, in ten days for 87% of the strains, and in 14 days for 100% of the strains. The turnaround time for NRA is lower than that of the direct proportion method, which can take upto 40 days to give a final result[1].

By performing First-Line drug susceptibility testing, MDRTB was detected in a total of 37/101 (6.63%) and 35/101 (34.65%) by both Proportion method and Nitrate Reductase Assay respectively in a study done by Singh[9]. In A study bY Z-K Huang the accuracy of NRA ranged from 94.4% to 99.1% for all drugs tested. In this study, for samples with discordant results, sequencing of known drug resistance relevant genes was performed, and data showed that about 43% of the NRA results were supported by genotypic detection[10].

Results of meta –analysis of Martin et al about NRA suggest that the NRA is highly sensitive and specific for determining INH & RIF resistant TB in

both culture isolates and directly on clinical sputum specimen[11].

CONCLUSION:

As NRA is inexpensive it could be used to carry out the drug susceptibility testing in suspected MDR as well as in close contact(s) of MDR cases. Primary drug resistance can also be detected by this method if new cases are included. Thus being a rapid and simple method, NRA can be used in low resource settings. NRA described in the present study is simple and needs no additional equipment which are required in MGIT (Mycobacterial Growth Indicator Tube). The contamination of liquid media is a matter of concern. However, it was less in the present study probably due to the use of PANTA antimicrobial.

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