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Tuberculosis: Heading Towards Appropriate Diagnosis and Treatment

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ABSTRACT

Objective– To ensure that all the patients infected with tuberculosis get early appropriate treatment, it is necessary to differentiate between *Mycobacterium* tuberculosis complex (MTBc) and non-tubercular mycobacterial (NTM) infection, as both the strains have different lines of treatment. It is also important to identify multi-drug resistant tuberculosis (MDR-TB) early to provide appropriate treatment thereby reducing transmission and preventing drug resistance. Methods- Samples from patients suspected for pulmonary or extra-pulmonary tuberculosis was processed on liquid culture (mycobacterial growth indicator tube (MGIT-960). Positive isolates were differentiated as MTBc or NTM by TBcID kit identifying MPT-64 Antigen, then the MTBc isolates was further tested for the first line drugs for the early identification of MDR-TB isolates. Results- A total of 457 pulmonary and extra-pulmonary samples was processed, *Mycobacterium* was isolated from 77 samples, 42 isolates (55%) were MTBc and 35 (45%) were NTM. Out of 42 MTBc 23 (55%) isolates were sensitive to all the first line drugs and seven (16.6%) were MDR-TB strains. Average time for detection of drug sensitivity was eight days with Streptomycin, Isoniazid, Rifampicin and Ethambutol (SIRE) and 10 days with Pyrazinamide. Conclusion– Usage of BACTEC MGIT-960 system coupled with TBcID kit provided easy and early differentiation of MTBc from NTM, and drug sensitivity results which helped in planning early and appropriate treatment for MDR and NTM cases.

INTRODUCTION

India has the highest burden of tuberculosis globally accounting for around 1/5th of the total global cases, the situation is further worsened with highest cases of MDR tuberculosis [1] (i.e. the disease caused by the *mycobacterium* strain which is resistant to at least two first-line drugs isoniazid and rifampicin) and undiagnosed NTM infections. Microscopy cannot differentiate between MTBc and NTM, moreover, the treatment given to the patients with MTBc and NTM are different, therefore rapid isolation, detection, and discrimination of *mycobacterium* spp. is need of the hour for suitable management. Although mycobacterial species can be identified by the use of conventional biochemical methods, but these are time-consuming, tedious and require extensive safety precautions, whereas molecular methods used for the identification though very accurate and reliable, but require a specialized and sophisticated set up with expensive equipment and trained laboratory personnel. Therefore for resource- poor countries like India it is not easy to set a molecular laboratory. On the other hand automated methods (MGIT 960) are giving promising results with good sensitivity and specificity being less time to consume and proving cost-effective in resource-poor countries.

To spare patients from unnecessary treatment, in cases of NTM infection, it is important to identify them. MPT64 antigen is the key for their identification. MPT 64 Ag is secreted during its growth and is specific for MTBc (*M. tuberculosis*, *Mycobacterium Bovis* (subsp. *Bovis*, subsp. *caprae*, and subsp.*BCG*), *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium canettii*, *Mycobacterium mungi*, *and Mycobacterium pinnipedii*) [2].

With early diagnosis of MDR-TB, the outcomes of treatment are expected to improve. The present study was done to evaluate the accuracy and clinical usefulness of immune-chromatography test kit which is based on mouse monoclonal anti-MPT 64 to differentiate between MTBc and NTM in samples from patients with pulmonary and extra-pulmonary tuberculosis (EPTB).

Till date, no study has been conducted in the Himalayan region of India to evaluate MGIT 960 for the diagnosis of TB and drug susceptibility for both pulmonary and extra-pulmonary samples. So the present study was planned for first-line drug susceptibility testing of MTBc isolate and differentiation between MTBc & NTM by immune-chromatography assay.

MATERIALS AND METHODS

A total of 457 clinical samples were collected over a period of six months from patients suspected to have pulmonary or EPTB. Sputum samples, BAL and all body fluids (ascitic tap, pleural tap, urine, synovial fluid, drains, pus discharge, gastric aspirates, CSF), tissues and urine samples were collected in a sterile container and stored at 4^0C till processed, but not more than 48 hrs.

Preliminary diagnosis of samples was done by microscopy Ziehl–Neelsen (ZN) staining (decolourized both by alcohol and 20% sulphuric acid) followed by culture in BACTEC MGIT-960 system (Middlebrook 7H9). Immuno-chromatography test was done for positive isolates to differentiate between MTBc and NTM. MTBc isolates were further processed for first line drug sensitivity testing in liquid culture by BACTEC MGIT-960.

The samples were handled in Biosafety Level-2+ laboratory (PPE + N95 mask).

Samples received was digested, decontaminated and concentrated following the standard protocol [3]

The instrument positive tubes were confirmed for acid-fast bacilli by Z-N method then, 100µl (microliter) of broth from liquid culture was added to the well of TB Ag MPT 64 kit cassette. Results were read as positive when two red lines appeared within 15 min. at the control (C) site and the test (T) site. This was according to the literature insert, as per manufacturer instructions.

The instrument positive tubes were used for drug susceptibility testing within five days of the instrument positive. A tube that has been positive for more than five days was sub-cultured in a fresh MGIT tube and the procedure was repeated.

The MTBc growth tube was mixed well (vortexed) to break up clumps then dilution was done with normal saline up to 0.5 McFarland turbidity. Conc. of various drugs used was – streptomycin (STR)- 1µg/ml, isoniazid (INH)- 0.1µg/ml, rifampicin (RIF)- 1µg/ml, ethambutol (ETB)-5 µg/ml, pyrazinamide (PZA)- 100 µg/ml. The drugs were tested in two panels one with STR, INH, RIF, ETB along with the growth control (GC) without drug and the other panel was with PZA and GC. Both the panels had different dilutions, and the procedure followed was as per the protocol provided [3]. Drug susceptibility was reported when the growth control units reached 400 as indicated by the instrument.

RESULTS

Total of 457 samples were processed in the study, distribution of the sample type processed and their detection by various methods are shown in **Table**-1

Table no 1: Various samples processed in the study.

Samples	Total samples	Positive samples	Number of positive samples **(%)**		
			Z-N	L-J	MGIT 960
Sputum	82	23	12**(52.2)**	10**(43.7)**	23**(100)**
BAL	54	11	3**(27.2)**	7**(63.6)**	11**(100)**
Pus	76	9	5 **(55.5)**	6**(66.6)**	9**(100)**
Urine	45	6	3**(50)**	3**(50)**	6**(100)**
Pleural fluid	41	7	2(**28.5)**	4(**57.1)**	7**(100)**
CSF	37	5	2**(40)**	3**(60)**	4**(80)**
Ascitic fluid	18	3	2**(66.6)**	1**(33.3)**	3**(100)**
Knee aspirate	25	2	1**(50)**	0**(0)**	2**(100)**
Lymph node aspirate	31	6	4**(66.6)**	3**(50)**	5**(83.5)**
Ileal/colonic biopsy	24	2	0**(0)**	1**(50)**	2**(100)**
Bone marrow	10	1	0**(0)**	0**(0)**	1**(100)**
Skin biopsy	14	2	1**(50)**	1**(50)**	2**(100)**
Total	457	77	35**(45.4)**	39**(50.6)**	75**(97.4)**

Out of 457 samples77 samples were positive on culture both on solid and liquid media, for the growth of *Mycobacterium* spp., these 77 isolates were differentiated as MTBc & NTM by immune-chromatography assay identifying MPT64 Ag. Out of them, 42(55%) isolates were MTBc, and 35 (45%) isolates were NTM.

Antibiotic susceptibility testing was done for all the first-line drugs on 42 strains of MTBc isolated from both liquid and solid media, using BACTEC MGIT 960 system.

Concentration of various drugs used were - STR- 1µg/ml, INH- 0.1 µg/ml, RIF- 1 µg/ml, ETB-5 µg/ml, PZA- 100 µg/ml. Strains sensitive to all drugs were 23 (55%) and strains resistant to one or more drugs were 19 (45%)

Drug sensitivity pattern of first line drugs is shown in **Table 2**.

Table no. 2: Number of stains sensitive to various first line drugs

Drugs	No. of resistant strains	No. of sensitive Strains
Streptomycin	10	32
Isoniazid	14	28
Rifampicin	8	34
Ethambutol	6	36
Pyrazinamide	7	35

Mono-resistance pattern in first line drugs is shown in **Table 3** and poly-resistance pattern in first line drugs is shown in **Table 4.**

Table no. 3: Number and percentage of monoresistant strains

Drugs	No. of Strains	% of Resistant strains
Streptomycin	1	2.38
Isoniazid	5	11.9
Rifampicin	0	0
Ethambutol	0	0
Pyrazinamide	2	4.76

Table no. 4: Number and percentage of poly-resistant strains

Drug combination	No. of resistant cases	% of resistance
STR + INH	0	0%
STR + RMP	0	0%
STR + ETB	0	0%
STR + PZA	0	0%
INH + RMP	0	0%
INH + ETB	0	0%
INH + PZA	1	2.40%
RMP + ETB	1	2.40%
RMP + PZA	0	0%
ETB + PZA	1	2.40%
INH + RMP + ETB	0	0%
INH + RMP + STR	3	7.10%
INH + RMP+ ETB +STR	1	2.40%
INH + RMP+ ETB +STR +PZA	3	7.10%

Seven isolates were resistant to both drugs (INH and RIF). Distribution of MDR isolates is shown in **Table 5**. Out of the total seven MDR cases detected, two were new cases and five were with previous history of ATT.

Table no. 5: Distribution of MDR isolates.

	No. of cases	% of cases
MTBc cases	42	54.5
MDR cases	7	16.6
Old MDR cases	5	11.9
New MDR cases	2	4.7

Time for detection of sensitivity for SIRE drugs was eight days, Standard deviation (SD) was 2.04 days, median was eight days and mode was seven days. Minimum and maximum days for drug sensitivity results were four and 15 respectively.

Time for detection of sensitivity for pyrazinamide (PZA) was 10 days, SD was 1.5 days, median was 10 days and mode was 10 days. Sensitivity results were obtained within minimum of six and maximum of 13 days of strain inoculation.

DISCUSSION

Mycobacterium spp. is intrinsically resistant to many antibiotics, this owes to its physiology; as they have a lipid-rich cell wall which is impermeable to majority of drugs, and they grow slowly compared with other bacteria and can be dormant for a long time resulting in slow killing. Mycobacterial species are intracellular pathogens; therefore they are inaccessible to the agents that penetrate the mycobacterial cell wall poorly. Adding to this is the notorious ability of the organism to develop resistance early. Therefore combination of various drugs is required to overcome these obstacles and prevent emergence of resistance when on therapy. Above all is the concern for compliance as the response of mycobacterial infections to chemotherapy is slow, and therefore long treatment for months to years is required. All these factors are responsible for the special concern given particularly to the drugs and treatment of *Mycobacterium* infections.

Isoniazid (INH), Rifampicin (or other Rifamycin), Pyrazinamide, Ethambutol, and Streptomycin are the five first-line drugs used for the treatment of tuberculosis. Globally, the emergence of MDR-TB is a major threat to control tuberculosis.

Resistance to the first line ATT is increasing globally primarily due to poorly managed TB patients, the treatment of the patients is often inconsistent or partial, as the symptoms of the patients improve and they do not take the treatment regularly for the required period, because health care workers, at times, prescribe the wrong treatment regimens, or because the drug supply is unreliable or erratic.

The increase in the incidence of MDR-TB and the emergence of extensively drug-resistant TB is becoming a tremendous challenge for the global efforts to combat tuberculosis. Rapid methods like MGIT-960 enables early diagnosis & accurate susceptibility testing to first line drugs SIRE and for pyrazinamide, which is critical for the early diagnosis of MDR-TB and initiation of effective regimes.

Various studies [4,5,6,7] analysing the performance of immune-chromatography techniques concluded that TBcID test is a single step, rapid test (15 min), it is a simple test that does not require any sample preparation or any instrumentation, it is less expensive and the literature had reported 100% specificity and sensitivity of the ICT kit.

Though literature [8,9] have reported occasional false positive results with TBcID kit but rapidity with high specificity and sensitivity of the assay have significant implications in controlling tuberculosis. Thereby facilitating appropriate treatment and reducing the chance for further transmission, and this outweighs the occasional false positive reports.

The recovery rate of NTM in this study among positive samples is 45% and this rate falls within the 20-80% reported globally [10]; their recognition is also important to control emergence the of resistant *mycobacterium* strains due to their increasing role as pathogens and different line of treatment.

India is placed among the four countries with largest number of estimated cases of MDR-TB, others being China, the Russian Federation and South Africa. To be successful in the management of tuberculosis, rapid identification of MDR-TB and NTM is universally acknowledged. Detecting resistance at the genetic level by molecular methods have though provided with promising results, but the presence of multiple resistance mechanisms for the

majority of anti-mycobacterial drugs has caused major hindrance, leaving the phenotypic susceptibility testing methods as the only reliable and rapid option. Among them, the use of liquid media is the best possibility available for rapid and reliable testing. The radiometric BACTEC 460TB method which was used previously with good results is now obsolete, due to an increasing concern about radioactivity and its disposal.

In our study, we got the drug sensitivity pattern for SIRE drugs in an average of eight days and for PZA in an average of 10 days. Similar rapidity was also observed by Telles et al.[11], Gaby E. Pfyffer et al. [12] and Claudio Scarparo et al. [13], while comparing with standard conventional techniques, in addition, the sensitivity done using MGIT 960 system has multiple advantages as it requires least amount of labour, it is easy to use, has high-capacity, is fully-automated and have a continuous monitoring system. MGIT-960 also provides us with the advantage of studying the sensitivity pattern of Pyrazinamide which cannot be done on solid media.

Primary and secondary resistance in our study is similar to the results reported in the literature [12,13] (Primary resistance is the resistance pattern seen in new patients who have not previously been exposed to anti-TB drugs [14].Secondary resistance is the resistance pattern in patients with a previous history of anti-TB treatment and is due to ineffective chemotherapy [14])

Study reported from China [15] showed comparatively higher resistant rate (34%, 64%, 32%, 20% for STR, INH, RMP, and ETB) as one of the high TB burden country. On the other hand, a six-year observational study [14] regarding the primary resistant pattern in first line drugs as STR - 6.84%, INH - 17.17%, RIF - 5.28% and ETB – 4%. The resistance rate of 10% was comparatively much less than our results probably because the area of study (Turkey) is not reported to be one with high resistance rate in *Mycobacterium tuberculosis* strains.

Resistant pattern reported as mono-resistance refers to the resistance to a single first-line drug, and poly-resistance refers to the resistance to two or more first-line drugs. Patients who are infected with mono- or poly-resistant strains have been associated increased risk of treatment failure while on standardized short-course chemotherapy and further they acquire resistance, leading to the development of MDR-TB.

In our study, 20% of the total isolates had mono-resistance, 23% of the total isolates had poly resistance and 7% isolates were resistant to all the five drugs tested

These results were supported by a multicentric study by [16] and Mycal Pereira et al.[17]

Mono resistant pattern of our study further supplements the reporting criteria of GeneXpert (latest and rapid in reporting MDR strains [18]) as no strain is rifampicin-monoresistant.

The emergence of MDR-TB represents a major threat to control tuberculosis, as it has been shown that the major cause of MDR strains is the result of poor implementation of tuberculosis control strategies[19] In tuberculosis control program, it is very important to monitor drug sensitivity patterns in individuals as well as in the community in patients with chronic tuberculosis. Knowing the drug resistance patterns in a particular community is of great epidemiological significance as it is an indicator of the existence and prevalence of primary and acquired drug resistance in the locality. This data is, therefore, essential to evaluate the quality of the tuberculosis control programs and it also gives way for recognition of patients who need special treatment and isolation conditions.

Out of the 42 strains tested for first line drug sensitivity seven strains were resistant to both RMP and INH (MDR) i.e. total 17%. Among them, five (11.5%) were in old cases and two (4.7%) were in new cases.

The Global Project on Anti-Tuberculosis Drug Resistance Surveillance [20], has reported that the median prevalence of primary drug resistance i.e. MDR in strains isolated from new cases was only 1% (range 0-14.1%), whereas the median prevalence of acquired drug resistance i.e. MDR in previously treated cases was 9.3% (range 0-48.2%). Our results are within this range. Literature [15, 21, 22] has also reported MDR-TB ranging from 11.4% in new cases, and 19.1% in previously treated cases, to total MDR –TB 3.92% and 14% respectively.

Both primary and acquired drug resistance are increasing as a result of known factors like the treatment is taken inadequately, the quality of drugs used is substandard, there is use of inappropriate drug combination preparations, or “monotherapy” and noncompliance on part of patient. Noncompliance is due to number of reasons, major being the side effects of the anti-TB drugs and early symptomatic relief, others such as poverty, gender discrimination, and homelessness also compel patients to either discontinue treatment or to take the drugs irregularly and erratically. Delay in the relief of symptoms also affects the patient’s psyche

and they forced to shop around for other options for treatment. Health care systems also contribute to this problem - Despite DOTS policy, in 119 countries globally, it is estimated that only 40% of the TB cases are usually notified, suggesting that a large proportion of TB cases which are unreported are being managed in the private sectors [23]. Unfortunately, studies have shown that private practitioners do not necessarily prescribe the standard therapeutic treatment guidelines for tuberculosis. They often offer inappropriate and expensive treatment regimes [24].

The present study first from its region provided rapidly the basic information regarding whether the acid fast bacilli is MTBc or NTM and thereby could help to start early and appropriate treatment. Early drug sensitivity results further contributed by identifying MDR strains. This could ensure effective and appropriate management of tuberculosis which could minimize the development of resistance in ATTs. Therefore the surveillance of resistance pattern in ATTs is an essential tool to monitor the effectiveness of TB control programs and thereby provide us an ignition to improve our national and global TB control efforts.

CONCLUSION

Immuno-chromatography used for differentiating MTBc from NTM's is easy and rapid (results obtained in 15min.) method with committable results, therefore beneficial to the patient as the treatment for both the groups is different. Early detection of resistance pattern of first line drugs with an additional advantage of PZA sensitivity testing (which is not recommended on solid media) helps to start early effective treatment in patients and to evaluate the quality of the tuberculosis control program as newer effective anti-TB drugs are still a distant dream.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

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