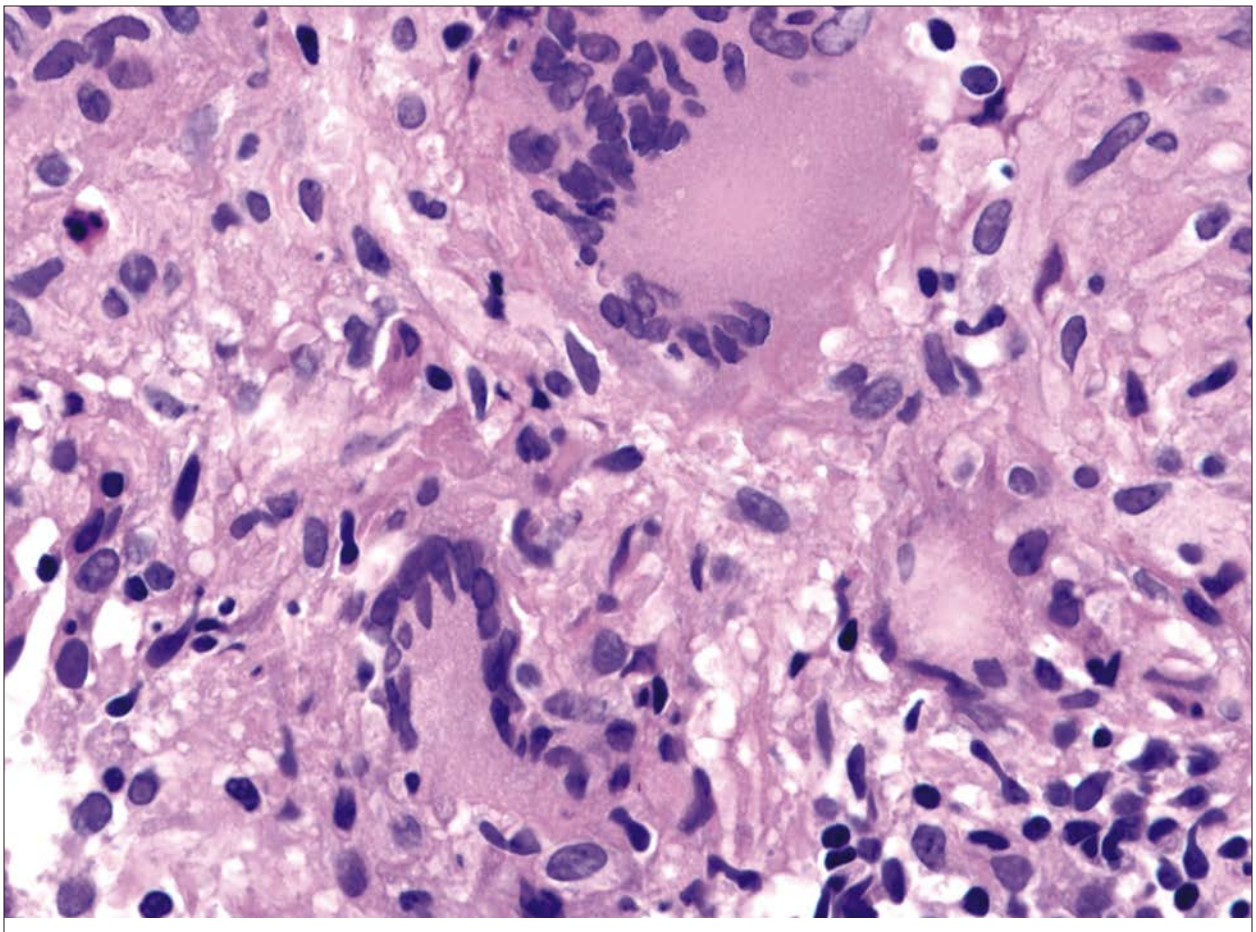

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Diagnostic Utility of Capilia TB Assay for Identification of *Mycobacterium tuberculosis* Complex

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ABSTRACT

Culture for *Mycobacterium tuberculosis* (*M. tuberculosis*) is important for tuberculosis control, so is the need for a rapid, simple and inexpensive identification test for mycobacterial culture isolates. The laboratories under Revised National Tuberculosis Control Programmes (RNTCP) in India are being scaled up by the introduction of rapid broth based culture systems. Laboratory mycobacterial culture isolates have to be further identified as *M. tuberculosis* or Non Tuberculous Mycobacteria (NTM) since accurate identification of *M. tuberculosis* complex is mandatory for appropriate diagnosis and treatment of tuberculosis. Biochemical tests are slow and sometimes require subcultures, as *M. tuberculosis* complex take weeks to grow. Capilia TB test is an immumno-chromatographic assay using monoclonal antibodies to detect MPB64 antigen/protein which is specific for *M. tuberculosis* complex (MTC). Therefore, the usefulness of Capilia TB test for culture confirmation of *M. tuberculosis* complex was evaluated in 75 mycobacteria positive clinical isolates by comparing it with conventional biochemical identification tests. The overall sensitivity and specificity was found to be 96.7 and 100%, respectively. The turn-around time for capilia ranged from 9-16 days as compared to biochemical identification which was 30-70 days. Capilia TB test is simple to perform and provides rapid confirmation of *M. tuberculosis* complex with minimal investment in terms of infrastructure, human labour and expertise. Laboratories using liquid culture may consider Capilia TB for rapid identification of *M. tuberculosis* complex.

Key words: Non-tuberculous mycobacteria, *M. tuberculosis*, biochemical identification, immuno-chromatographic assay, liquid culture system, anti-tubercular treatment

INTRODUCTION

Globally, prevalence of tuberculosis (TB) has reached epidemic levels with ever increasing proportion of Multi-Drug Resistant (MDR) and more recently, extensively drug resistant tuberculosis (XDR TB). Tuberculosis is mainly caused by members of *Mycobacterium tuberculosis* (*M. tuberculosis*) complex and some Non-Tuberculous Mycobacterium (NTM). Therefore, culture of mycobacterium with identification and drug sensitivity testing (DST) is mandatory for appropriate diagnosis and treatment of tuberculosis (WHO, 2009; Nikalje and Mudassar, 2011).

In developing countries, mycobacterium cultures and drug sensitivity tests are mainly done on solid media. These labs use biochemical tests for differentiation of *M. tuberculosis* complex from NTM. Biochemical tests sometimes require sub culturing which takes several days or weeks of incubation until sufficient growth is observed. The process increases the turnaround time for reporting. Molecular methods for identification such as DNA probes and nucleic acid amplification are rapid but expensive for routine use. Also these tests require expensive infrastructure and technical expertise. There has been significant change in management of tuberculosis since pre chemotherapeutic era to date (Imam *et al.*, 2010).

Recently under RNTCP laboratories are increasingly implementing rapid broth based culture systems facilitated by Foundation for Innovative Newer Diagnostics (FIND) due to reduction in cost of equipment and supplies (WHO, 2007). The program recommends the use of liquid culture systems for all Intermediate Reference Laboratories (IRLs) and National reference laboratories (NRLs).

The MGIT 960 (Mycobacterium growth indicator tube BACTEC MGIT 960, Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) culture system has been evaluated and has shown the potential to reduce the delay in diagnosis and reporting of drug resistant cases of TB (Alcaide *et al.*, 2000; Cruciani *et al.*, 2004; Somoskovi and Magyar, 1999). However, for these systems, a rapid and accurate test for identification of culture isolates is required; otherwise the purpose of introducing these systems will not be fulfilled.

Capilia TB (TAUNS, Japan) is a rapid test for differentiating *M. tuberculosis* complex from NTM. The technology is based on immuno-chromatographic assay using monoclonal antibodies to detect MPB64, one of the predominant proteins secreted by *M. tuberculosis* complex strains with exception of some *M. bovis* and Bacillus Calmette Guerin (Abe *et al.*, 1999). The test is performed directly from the liquid cultures or from resuspended colonies from solid media. As the sample flows laterally through the nitrocellulose membrane anti mpb64 mouse monoclonal antibodies conjugated with gold colloidal bind to MPB64 antigen if present in the sample producing red purple band within 15 minutes. The capilia test performs rapid identification of the *M. tuberculosis* complex with minimum additional human and laboratory inputs (Abe *et al.*, 1999; Hillemann *et al.*, 2005; Ngamlert *et al.*, 2009; Wang *et al.*, 2007; Shen *et al.*, 2009).

In the present study, the performance of capilia was evaluated by comparing it with routine biochemical identification tests. We also made an attempt to correlate results of identification with presence of cords in cultures.

MATERIAL AND METHODS

Sample collection: Samples submitted to the Microbiology laboratory, Lala Ram Sarup Institute of Tuberculosis and Respiratory Disease (LRSI of TB and RD) for mycobacterial culture in MGIT 960 from January 2010 to June 2010 were considered for the study.

Seventy five samples (72 sputum and 3 pus) from 68 patients were included in the study. The patients were asked to submit a repeat sample in case the Capilia TB test turned out negative. Out of 11 Capilia negative cases, 5 patients submitted second sample, 1 patient submitted a second and third sample.

Sample processing and inoculation: Sputum samples were homogenized and decontaminated with NaLC-4% NaOH, 2.9% citrate (N acetyl L cysteine-sodium hydroxide citrate) method. Equal

volume of NALC-NaOH citrate solution was added to the sample. After mixing and incubation at room temperature for 15 min, specimens were concentrated at 3000 g for 15 min. Supernatant was decanted and pellet was suspended in 2 mL (pH-6.8) of Phosphate Buffer Saline (PBS). A suspension of 500 µL and 100 µL was used to inoculate MGIT 960 tubes and Lowenstein Jensen (LJ) slants, respectively.

Biochemical identification: The LJ slants were observed weekly till 8 weeks of growth. Growth was monitored for colony morphology, pigmentation and stained by Ziehl Neelsan (ZN) method to date it for presence of acid fast bacilli (AFB). The LJ positive for mycobacterium was subjected to conventional biochemical differentiation by niacin, nitrate and semi-quantitative catalase tests (Kent and Kubica, 1985).

Observation of cords and capilia results: Smears were prepared from all tubes flagged positive by MGIT 960 instrument and stained by (ZN) method to examine AFB and presence of serpentine cords. For Capilia TB assay 100 µl of positive MGIT960 broth culture was placed onto specimen area. Incubation was done for 15 min followed by observation of purplish-reddish colour change.

RESULTS

A total of 75 isolates were obtained in the study. Capilia TB identified 58 isolates as *M. tuberculosis* complex and 17 isolates as NTM. All 58 isolates identified as *M. tuberculosis* by capilia were confirmed by biochemical identification as well. No discrepancy was found among the two. Two out of 17 isolates identified as non-tuberculous mycobacterium by capilia were characterized as *M. tuberculosis* by biochemicals. Sensitivity and specificity of Capilia TB for *M. tuberculosis* complex identification was 96.7% and 100%, respectively (Table 1). Among these capilia gave a positive resultfor one patient upon repeat testing from solid media growth.

The turn around time from specimen receipt to reporting of the test was calculated. The median time from specimen receipt to identification of *M. tuberculosis* complex by capilia was 16 days for smear negative samples 14 days for samples with 1+ smear and 9 days for samples with 2+ and 3+ smears. For biochemical identification the time ranged from 30-70 days.

Isolates from 9 patients were identified as non-tuberculous mycobacterium by both Capilia and biochemical identification. Out of these, eight were either relapse or failure cases and one patient was a new case. The clinical details of these patients are depicted in Table 2. The patients suffered from symptoms suggestive of tuberculosis from last 3-4 years and some had history of repeated Anti tubercular treatment (ATT). The prior culture identification details are not available to say if *M. tuberculosis* complex was diagnosed at any point of time in the previous years. On smear microscopy eight out of nine cultures showed absence of cords whereas cords could be seen in all capilia positive for *M. tuberculosis* complex.

Table 1: Comparison of results obtained by capilia TB assay and biochemical identification tests

	Biochemical identification		
Capila TB	MTBC	NTM	Total
Positive	58	nil	58
Negative	2*	15	17
Total	60	15	75

* Sample from 1 of the discordant isolates, negative for capilia was repeated and turned to be positive on repeat testing from solid media

Table 2: Clinical details of patients diagnosed as non-tuberculous mycobacterium by both Capilia and biochemical identification

No. of samples received	Age/sex	Smear	Days for positivity of	Presence/absence of cords	Clinical details
Patient 1 (2 samples)	19/M	Negative	4, 7	Absent	Failure case, sick for last 4 years,
Patient 2	41/M	3+	3, 3	Absent	Sick for the last three years
Patient 3 (2 samples)	63/M	Negative	4, 4, 3	Absent	New case, fever for last 15 days,
Patient 4	44/F	Negative,	5, 4	Absent	History of tuberculosis 15 years back,
Patient 5 (3 samples)	13/M	Negative	7, 9, 15	Present	Failure case, under categoryII
Patient 6 (1 sample)	50/F	1+	3	Absent	Anti tubercular treatment for 4-5 years. Repeated courses including CategoryI and Category II. Resistant to all I line drugs and was mentioned as extensively drug resistant in a report from private lab
Patient 7 (1 sample)	60/F	2+	3	Absent	Failure case
Patient 8 (1 sample)	52/M	Negative	7	Absent	History of category and category II treatment
Patient 9 (1 sample)	60/M	2+	3	Absent	Failure case on retreatment regimen

DISCUSSION

The role of culture is becoming important for TB control in India with many new labs being set up under the RNTCP for broth basea culture and drug susceptibility testing of *M. tuberculosis*. Therefore, there is a need for rapid and simple identification test for mycobacterial culture isolates. It was found that the Capilia TB test for identification of *M. tuberculosis* complex from broth culture performs with acceptable sensitivity, specificity and accelerates time to *M. tuberculosis* complex confirmation. The test was found to have excellent positive predictive value as no false positive results were obtained. Previous studies have also evaluated Capilia TB test with sensitivity ranging from 92.4%-99.6% and specificity ranging from 98-100% (Hasegawa *et al*., 2003; Hillemann *et al*., 2005; Hirano *et al*., 2005; Muyoyeta *et al*., 2010; Ngamlert *et al*., 2009; Shen *et al*., 2009; Wang *et al*., 2007).

M. tuberculosis appears as cords in liquid culture whereas NTM usually does not bundle as cords. All the *M. tuberculosis* complex by capilia were seen as cords and 8 out of 9 non-tuberculous mycobacterium showed absence of cords. Two isolates gave false negative results which could be because of low bacillary load in liquid medium as one capilia negative case gave a positive result on repeat testing from solid media growth. Muyoyeta *et al*. (2010) also found false-negative result for one *M. tuberculosis* complex culture isolate when GenoType Mycobacterium CM assay was used as the gold standard. False negative results due to unique mutations in MPB64 gene have been reported which necessitates validation in diverse settings (Hasegawa *et al*., 2003; Hirano *et al*., 2005; Ngamlert *et al*., 2009). For all capilia negative samples, smear morphology for presence/absence of serpentine cords, repeating capilia from subcultures and correlation with biochemical tests/molecular method should be considered before reporting capilia results.

Of nine patients identified as non-tuberculous mycobacteria by both Capilia and biochemical identification eight had taken anti tubercular treatment more than once. The exact disease burden of non-tuberculous mycobacterium infections still remains unclear in India. These infections are under diagnosed and hence underreported due to lack of culture and identification facilities. A prompt reporting of these would ensure appropriate regime in such cases and would prevent unnecessary and inappropriate treatment with anti tubercular treatment.

CONCLUSION

As the study includes limited number of samples, more studies need to be done in different settings as genotypic difference among strains may affect the performance of the test. Capilia TB test is rapid and easy as it provides rapid confirmation of *M. tuberculosis* complex with minimal additional human or lab resources. Introduction of MGIT 960 has reduced the time for culture and drug susceptibility testing and a test like Capilia TB could further facilitate prompt management of tuberculosis cases.

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