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Validity of a CB-NAAT assay in diagnosing tuberculosis in comparison to culture: A study from an urban area of South India

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1. Introduction

The world today is known for its advancements in technology in all social sectors, most importantly in the health sector and specifically in the field of Mycobacteriology. Clinically suspected TB cases are normally tested for the presence of *Mycobacterium tuberculosis* in appropriate samples by laboratory diagnostic methods. Conventionally, Acid fast bacilli (AFB) smear microscopy and the culture methods are employed for the diagnosis. Fluorescence microscopy of Auramine-O stained smears and liquid culture using tools like the BACTEC™ "Mycobacterial Growth Indicator Tube 960" (MGIT 960; Becton-Dickinson, Sparks, MD, USA) are the most commonly used conventional methods. Culture method is the most sensitive and specific method for the detection of *Mycobacterium tuberculosis*. Despite this, cultures are highly prone to contamination and the process can still take several days and does require expensive equipment, strict biosafety practices and well trained technical staff [1].

Among the currently available Nucleic Acid Amplification Tests (NAAT), the Xpert MTB/RIF Assay (CB-NAAT), the LINE Probe Assay (LPA) and the Loop-Mediated Isothermal Amplification (LAMP) are endorsed by WHO for *in vitro* diagnosis of TB. The GeneXpert® system powered by the Cepheid Innovations, for the CB-NAAT (Cartridge based), is an automated, semi-quantitative, hemi-nested, real-time PCR used for the simultaneous detection of the MTB complex and its rifampicin (RIF) resistance pattern associated with the mutation in the *rpoB* gene, in clinical samples with a 2 h turnaround time [2].

The present study was carried out to assess the performance of CB-NAAT (Cepheid GeneXpert®) system for the diagnosis of MTB in both pulmonary and extrapulmonary specimens, within the demographic area of Mangalore, in South Karnataka.

2. Materials and methods

The study was approved by the Institutional Ethics Committee of Kasturba Medical College, Mangalore. This cross-sectional study was conducted at the Department of Microbiology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, in Dakshina Kannada District of Karnataka State in India, over a period of 31 months from June 2016 to December 2018. The Department of Microbiology consists of Designated Microscopy Center as per the Revised National Tuberculosis Control Program (RNTCP) under the DOTS (Directly Observed Treatment, Short course), which caters to attached tertiary care hospitals of the study Institute. The health care provided in these hospitals includes people from neighboring Districts of Karnataka and Kerala States. Pulmonary & extrapulmonary specimens received at the Microbiology laboratory, in sterile containers, from the clinically suspected tuberculosis patients were used in the study. Saliva, blood, urine and stool samples were excluded from the study.

The samples received at the laboratory were divided into three portions, one part each was used for AFB direct smear preparation, CB-NAAT and MGIT liquid culture, respectively. Direct and concentrated smears were prepared, stained using the Fluorochrome acid-fast staining method, with the Auramine-O fluorescent stain and screened as per the guidelines [3]. A smear was reported positive if either the direct or the concentrated smear showed the presence of AFB. The NALC-NaOH method [4] was used for the sample digestion and decontamination. The concentrates were cultured on to BD BBL™ MGIT™ Mycobacteria Growth Indicator Tube using the BACTEC™ MGIT™ 320 system. Cultures were incubated for up to 8 weeks to confirm the negativity of *Mycobacteria* in the sample. If positive, the culture was subjected to a rapid immunological ID test using the BD MGIT™ TBc ID test device to differentiate MTB from *Mycobacterium* genus. CB-NAAT was done using

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the Cepheid GeneXpert® system, according to the manufacturer's instructions [2]. At the end of the test, the result was reported as MTB detected or MTB not detected along with Rifampin (RIF) resistance status.

The collected data were coded and entered onto Statistical Package for Social Sciences (IBM SPSS Statistics for Windows) version 25.0. Armonk, NY:IBM Corp. Results were expressed as proportions using tables. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were calculated, by considering the liquid culture results as the gold standard. For comparison across the CB-NAAT and culture groups, chi-square test was used and a p value of <0.05 was considered as statistically significant.

3. Results

The samples received from suspected TB patients were from different localities within and around the urban area of Mangalore, which included Bantwal, Puttur, Moodbidri, Belthangady, Sullia of Dakshina Kannada district, Udupi, Hassan, Chikmagalur districts of Karnataka and Kasaragod and Kannur districts of Kerala. Samples obtained from 831 patients were included in the study of which 507 (61.01%) were males, 324 (38.99%) were females. The mean age of the patients was 52.01. The pulmonary samples (n = 682) included in the study were Bronchial Alveolar Lavage (BAL) [N = 591], sputum [N = 84], endotracheal (ET) aspirate [n = 6], and bronchial biopsy [n = 1] (Fig. 1). The extrapulmonary samples (n = 149) were body fluids [n = 75], tissues [n = 34], pus [n = 30], lymph node aspirates [n = 8] and gastric lavage [n = 2] (Fig. 2).

Smears were positive in 72 patient samples, which consisted of 56 pulmonary and 16 extra-pulmonary cases. The true positive occurrence with the use of smear microscopy was 95.83%. A positive culture growth was observed in 148 pulmonary and 32 extrapulmonary samples. The rapid ID test had identified 122 cultures as MTB (95 pulmonary and 27 extrapulmonary) and the rest 58 (53 pulmonary and 5 extrapulmonary) as Genus *Mycobacterium* species. Table 1

The GeneXpert® system detected the presence of MTB in 106 pulmonary and 30 extrapulmonary samples. The true positive rate of detection of MTB by CB-NAAT was found to be 75.74%. Five cases of RIF resistance were also detected. 30 samples positive by CB-NAAT had grown Genus *Mycobacterium* species in culture. Additionally, CB-NAAT detected MTB in three samples which were smear positive and culture negative. CB-NAAT also detected MTB in 61 smear positive and culture positive samples, 42 smear negative and culture positive samples, and 33 smear negative and culture negative samples.

The sensitivity, specificity, PPV and NPV of smear microscopy were 38.33, 99.54, 95.83 and 85.38% respectively and that of the CB-NAAT assay is tabulated in Table 2.

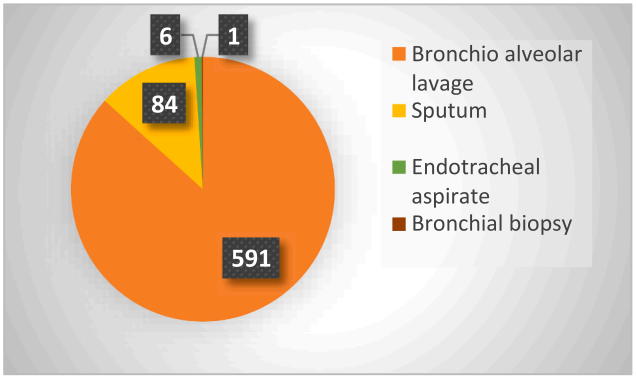

Fig. 1. Distribution of pulmonary samples.

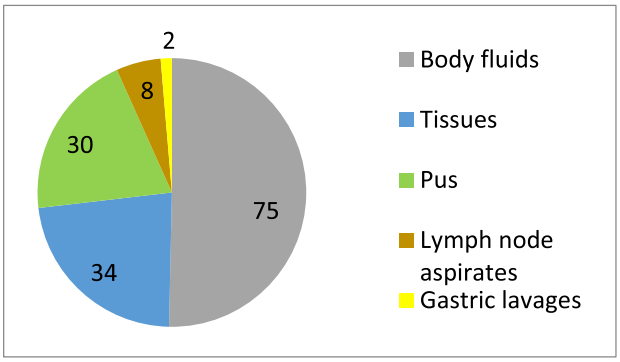

Fig. 2. Distribution of extrapulmonary samples.

Table 1
AFB smear comparison with culture.

	Culture Positive	Culture Negative
Smear Positive	69	3
Smear Negative	111	648

4. Discussion

In spite of rapid strides in diagnosis and treatment, TB still continues to be a menace in many developing countries, including India. TB is among the 10 cardinal causes of mortality across the globe. The fight against TB has definitely given notable results. In the last 17 years, about 53 million lives were redeemed from the clutches of TB, mainly through timely diagnosis and effective treatment. TB incidence and mortality rate is at 2 and 3% per year respectively [5]. The major hindrance in the combat against TB is the lack of early diagnosis and appropriate and timely treatment. Programs like RNTCP and DOTS are efficiently confronting this issue with high priority, thus decreasing complications towards a great extent. Additionally, since 2003, Foundation for Innovative Diagnostics (FIND) has been working towards betterments in TB diagnostics and has improved the access to new diagnostic tools, by all countries, impartially [6].

Our study included the assessment of performance of GeneXpert® system in detecting MTB infection among the patients in and around the district of DK in Karnataka. Such an evaluation is the first of its kind in this area and thus the comparison is mainly with other studies across India [7] or abroad [8–12]. There are a few studies which have compared the performance of CB-NAAT for both pulmonary and extrapulmonary samples [8]. Most of the studies focus either on pulmonary samples [7,9,10] or on extrapulmonary samples alone [11,12]. The positivity in males was comparatively higher to that of females, in India, as well as abroad, reflecting the fact that males are more often infected by TB than females [13]. A study based on knowledge of TB reported that men sought better healthcare while women tend to self-medicate [14]. Earlier studies also reported that, apart from socio-economic and cultural factors, there may be certain biological gender factors that give rise to this sexual bias in TB [13].

The sensitivity and PPV of the CB-NAAT in our study was lower when compared to previous studies (91.4 and 86.5% respectively) [8]. The lower sensitivity can be attributed to the 19 false negatives (16 BAL) obtained with the CB-NAAT. BAL is known to have a lower sensitivity for the detection of MTB by CB-NAAT [10]. Another reason could be the very low load of the organism in the sample, lower than the detection limit of CB-NAAT (131 CFU/mL of sample) [2]. The lower PPV can be attributed to the 33 positive cases obtained by CB-NAAT which failed to grow in culture. The inability to grow in culture and ability to be detected by CB-NAAT may have been due to the paucibacillary nature of extrapulmonary specimens. It may also be due to the treated cases where

Table 2
The sensitivity, specificity, PPV and NPV of CB-NAAT in comparison to culture.

SAMPLES (n = 831)	SENSITIVITY (%)	SENSITIVITY (%)		SPECIFICITY (%)	PPV (%)	NPV (%)
		S + C+*	S−C+#			
PULMONARY (n = 682)	82.11	97.96	65.21	94.76	73.58	96.75
EXTRA-PULMONARY (n = 149)	92.59	100	85.71	95.73	83.33	98.25
OVER ALL	84.45	98.39	70	94.93	75.73	97.02

* Smear positive and culture positive.
# Smear negative and culture positive.

even the dead bacilli were detected by the CB-NAAT or the cases where the use of NALC-NaOH treatment for decontamination of the samples proved excessively harsh resulting in no growth. The specificity and NPV were almost at par with the previous studies (93 and 95.6% respectively) [8]. In the case of pulmonary samples, the total sensitivity was similar to other studies (79.5%) and was lesser than that of certain other studies (95.7%) [9,10]. While the NPV and the sensitivity for smear positive and culture positive samples were comparable to former studies (94–98.1% and 88.9–99.2% respectively), the specificity, PPV and sensitivity for smear negative and culture positive samples were lower than previous studies (99.6–100%, 99–100% and 73.1–77.7% respectively) [9,10]. In the case of extrapulmonary samples, the total sensitivity, sensitivity for smear negative and culture positive samples, smear positive and culture positive samples and NPV were higher than that of former studies (71–83, 66, 95 and 90% respectively) [11,12]. These differences observed in terms of extrapulmonary samples can be accounted to the lower number of extrapulmonary samples included in the study. The specificity and PPV were similar to previous studies (95 and 83% respectively) [11,12].

In the present study, CB-NAAT was found to be better than that of smear microscopy (with a difference of above 45% in terms of sensitivity), as observed by the earlier studies [15]. Adding to its advantage, this test is rapid, require minimal training of personnel and lower biosafety level (compared to culture) [16]. The CB-NAAT is used for the rapid detection of MTB in the samples. In settings where the incidence of non-tuberculous *Mycobacteria* (NTM) exceeds that of MTB, this assay may not be a success as a rapid diagnostic tool. Our study has shown a significant presence of probable NTM in cultures (58 out of 180 positive cultures). With growing incidence of NTM in many areas in India [17], CB-NAAT needs to be made more useful by including a distinguishable detection of NTM. Apart from this, the CB-NAAT is also comparatively disadvantageous in terms of its cost, shelf-life of cartridges, requirement of continued power supply and the need for the periodic servicing and calibration of the equipment [18]. This study could have been strengthened if all the mycobacteria isolates in our study were identified to species level.

5. Conclusion

This study could therefore, successfully favor the use of CB-NAAT, using the Cepheid GeneXpert® system, as a rapid method for the detection of MTB alone. Further research is required for development of a better diagnostic method that can simultaneously distinguish MTB and NTM, thus rendering to detect and treat the increasing incidence of infections caused by NTM.

Ethical Statement

The study "Validity of a CB-NAAT assay in diagnosing Pulmonary and Extrapulmonary Tuberculosis in comparison to culture: A study from an urban area of South India" was conducted after the approval of the Institutional Ethical committee. There was no patient involved. The study was conducted on the coded blinded left over sample.

CRediT authorship contribution statement

Aishwarya Raj: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft. **Shrikala Baliga:** Funding acquisition, Methodology, Resources, Supervision, Validation, Visualization, Writing - review & editing. **M. Suchitra Shenoy:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **B. Dhanashree:** Data curation, Formal analysis, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **P. Prasanna Mithra:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Smitha K. Nambiar:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Leesha Sharon:** Formal analysis, Investigation, Validation, Visualization, Writing - original draft.

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