Rapid detection of *Mycobacterium tuberculosis* and rifampicin resistance in extrapulmonary tuberculosis and sputum smear-negative pulmonary suspects using Xpert MTB/RIF

Irfan Ullah,† Arshad Javaid,† Haleema Masud, Mazhar Ali, Anila Basit, Waqas Ahmad, Faisal Younis, Rehana Yasmin, Afsar Khan, Abdul Jabbar, Masroor Husain and Zahid Ahmad Butt

Abstract

**Background.** Tuberculosis (TB) is a serious public health problem in developing countries such as Pakistan. Rapid diagnosis of TB and detection of drug resistance are very important for timely and appropriate management of multidrug-resistant TB (MDR-TB).

**Objective.** The purpose of this study was to determine the diagnostic efficacy of the Xpert MTB/RIF assay for rapid diagnosis of TB and detection of rifampicin (RIF) resistance in extrapulmonary and smear-negative pulmonary TB suspects.

**Methods.** A total of 98 bronchoalveolar lavage fluid (BALF) and 168 extrapulmonary specimens were processed by Xpert MTB/RIF. Culture results are considered as the gold standard for diagnosis of TB, and drug susceptibility testing for detection of RIF resistance. Diagnostic efficacy was measured in terms of sensitivity, specificity and positive and negative predictive values.

**Results.** The Xpert MTB/RIF assay detected 40 (40.8 %) of 98 BALF of presumptive pulmonary TB and 60 (35.7 %) of 168 extrapulmonary specimens. Sensitivity and specificity of the Xpert MTB/RIF assay for detection of TB was 86 and 88.4 %, respectively. The positive predictive value was 71.5 % while negative predictive value was 95.1 %.

**Conclusion.** The Xpert MTB/RIF assay is a rapid and simple technique with high sensitivity and specificity for diagnosing TB and detecting drug resistance in extrapulmonary and smear-negative TB cases.

INTRODUCTION

Tuberculosis (TB) is still a leading global public health issue, claiming the lives of 1.5 million individuals each year [1]. TB disproportionately affects people living in low- and lower middle-income countries, with almost 84 % of cases coming from only 20 countries [2]. Pakistan is among these top 20 countries contributing to the highest burden of TB and multi-drug-resistant (MDR) TB [2]. Despite several programmes, case detection rates are quite low (63 %) and a majority of patients remains undiagnosed and untreated [3]. The Government of Pakistan has introduced sputum smear tests at primary level in all hospital facilities in the country. However, sputum smear microscopy has low sensitivity, with a power of detecting only 50 % of active cases [4], which contributes to delayed diagnosis and thus continued transmission. Furthermore, the diagnostic challenge remains, as almost 20 % of cases of pulmonary TB in Pakistan are smear negative [3]. The rates of smear-negative pulmonary and extrapulmonary TB are on the rise globally [1]. Pakistan has also experienced an increase in the...
number of extrapulmonary TB patients who remain undiagnosed in the long term due to unavailability of appropriate diagnostic techniques. This delay in diagnosis and treatment initiation may be a contributing factor to excess mortality in people who have smear-negative pulmonary and extrapulmonary TB. Another delay experienced in Pakistan is that in initiation of appropriate treatment of MDR-TB, as confirmation of diagnosis and drug resistance detection takes up to 8 weeks. Therefore, such situations demand a quick, highly sensitive and economical method for both early detection of disease and determination of drug resistance status.

In recent years various molecular methods have been introduced for use with clinical specimens for rapid detection, including real-time PCR (GeneXpert MTB/RIF; Cepheid) and line probe assays (GenoTypeMTBDRPlus; Hain Life science and INNO LIPA Rif. TB; Innogenetics). Similarly, molecular assays have been developed to allow the rapid determination and identification of drug resistance in a single day from clinical specimens [5–9]. The Xpert MTB/RIF assay is a coheistic method for diagnosis of TB and fast detection of rifampicin (rif) resistance in clinical specimens that performs sample processing and semi-nested real-time PCR analysis in a single, hands-free step [5, 7]. The Xpert MTB/RIF test can be carried out in an almost fully automated way where assay is possible by PCR amplification of the Mycobacterium tuberculosis (MTB) rpoB gene (81 bp fragment) and subsequent probing of this region related to rif resistance. This test can normally be completed in less than 2 h [5, 7].

The World Health Organization (WHO) recommends use of Xpert MTB/RIF assay for national TB programmes in developing countries. However, few sites in Pakistan provide this service and sputum smears serve as the main screening test; these miss many smear-negative and extrapulmonary TB patients. We aimed to determine the diagnostic efficacy of this technique for both diagnosis of TB and detection of drug resistance for extrapulmonary and smear-negative pulmonary TB cases in Khyber Pakhtunkhwa (KPK), a highly TB-endemic area in Pakistan.

METHODS

Study setting
This study was carried out at the Programmatic Management of Drug resistant TB (PMDT) Unit in Lady Reading Hospital (LRH), Peshawar. It is the largest PMDT unit in KPK province, covering more than 30 TB-endemic districts in the region. Patients from tribal areas and neighbouring Afghanistan also seek care at this unit.

Study population
A total of 266 suspected TB patients were enrolled in the study. The enrolment period started in June 2014 and ended in May 2015. Inclusion of these TB suspects was based on the PMDT criteria for the Xpert MTB/RIF assay [10], also detailed in Table 1.

Clinical samples
Our clinical samples consisted of 98 bronchoalveolar lavage fluid (BALF) specimens from those patients who could not produce sputum, and 168 extrapulmonary specimens (pleural fluid, ascitic fluid, pericardial fluid) and pus specimens (lymph nodes). One specimen was collected from every enrolled case. The specimen was divided equally into two parts and each part was uniquely coded. One part was used for smear microscopy and Xpert MTB/RIF assay while the other part was used in culture and drug susceptibility testing (DST).

Xpert MTB/RIF assay
We evaluated the coded specimen by smear microscopy with Ziehl–Neelsen staining and Xpert MTB/RIF (Cepheid) to evaluate drug-resistant TB in patients. Smears were prepared by the auramine-rhodamine acid-fast staining technique. The MTB/RIF assay was done as described previously [5, 7, 11]. N-Acetyl-L-cysteine (NALC)–NaOH was added to the clinical specimens at a 3:1 ratio, for decontamination in a closed specimen [5, 7, 11]. The container was manually agitated for 15 min at room temperature. Two millilitres of inactivated specimen was transferred to the Xpert test cartridge and tested according to the manufacturer’s guide [5, 7].

Gold standard bacteriology and DST
The remainder of the samples were sent to the Provincial Reference TB Laboratory Hayatabad, Peshawar, for culture and DST. Non-sterile clinical specimens were processed using the standard mucolytic agent NALC–NaOH technique [12, 13]. Processing of specimens for culture was as described previously by us [14]. DST was performed after confirmation of MTB isolates. For resistance to rif (1 g ml⁻¹), the 1% proportional method was performed using Middlebrook7H10 medium (BBL, Becton Dickinson and Co.) as per standard guidelines [15] and described by Cantoni et al. [16]. MTB isolate H37RV, the laboratory strain, which is characteristically susceptible to anti-TB drugs, was used as a control.

Data collection and analysis
Data were obtained using both paper and electronic documentation systems of the PMDT unit. The socioeconomic (age, gender, residency, contacts),

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>1</td>
<td>Previously TB-treated cases with both positive and negative smears</td>
</tr>
<tr>
<td>2</td>
<td>Failure of Cat-I and Cat-II TB drugs</td>
</tr>
<tr>
<td>3</td>
<td>All smear-positive cases that remained positive by the end of the second month of TB treatment</td>
</tr>
<tr>
<td>4</td>
<td>TB/human immunodeficiency virus (HIV) co-infection cases</td>
</tr>
<tr>
<td>5</td>
<td>Seriously ill patients</td>
</tr>
<tr>
<td>6</td>
<td>Contacts of MDR-TB patients</td>
</tr>
</tbody>
</table>
microbiological (baseline sputum smear grading, microbiological culture status and DST results) and clinical data (pulmonary, extrapulmonary TB) of each patient were collected. A team of coordinators supervised the process of data collection to ensure completeness of the data entry.

Statistical analysis was performed using Microsoft Excel. Numerical variables were summarized with mean and standard deviation while categorical variables were summarized using frequencies and percentages. Diagnostic efficacy was measured in terms of sensitivity, specificity and positive and negative predictive values using two-by-two tables [17].

RESULTS
A total of 266 presumptive TB patients were enrolled in the study. Almost half of the patients were female (136, 51.1 %). The mean age of the patients was 34 years (sd=18.98), ranging from 3 to 80 years. More than half of the patients were aged 16–40 years (140, 52.6 %), 58 patients (21.8 %) were aged 41–60 years while 12 % (32) were above 60 years of age. A small proportion (36, 13.5 %) of the patients was below 16 years of age. Of the 266 patients included in this study, 160 (60.1 %) had a previous history of TB for which they had received treatment.

This study included 98 BALF and 168 extrapulmonary specimens. The extrapulmonary samples were drawn from ascitic fluid, cerebrospinal fluid (CSF), pus (from lymph nodes) and pericardial fluid (Table 2).

MTB was detected in 100 (37.59 %) specimens by the Xpert MTB/RIF assay, while the culture results showed the presence of MTB in 88 (33.08 %) specimens. Of 100 MTB-positive cases, as per Xpert MTB/RIF assay, 78 (78 %) were also culture positive while 18 (18 %) were culture negative and four (4 %) were reported to be contaminated. Among 266 specimens, concordance was found in 138 (62.7 %) MTB-negative specimens compared with 40 (87 %) MTB-positive specimens (Table 3).

Resistance to RIF was detected in 24 culture-positive specimens using DST. A higher proportion of previously treated cases was resistant (25 %) as compared to 3 % of newly diagnosed patients.

Table 2. Breakdown of pulmonary (BALF) and extrapulmonary samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>BALF</td>
<td>98</td>
<td>36.8</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>58</td>
<td>21.8</td>
</tr>
<tr>
<td>CSF (cerebrospinal fluid)</td>
<td>30</td>
<td>11.3</td>
</tr>
<tr>
<td>Pus (lymph nodes)</td>
<td>60</td>
<td>22.5</td>
</tr>
<tr>
<td>Pericardial fluid</td>
<td>20</td>
<td>7.5</td>
</tr>
<tr>
<td>Total</td>
<td>266</td>
<td>100</td>
</tr>
</tbody>
</table>

Diagnostic efficacy of Xpert MTB/RIF for TB detection

The overall sensitivity and specificity of the Xpert MTB/RIF assay for diagnosis of TB was 86 and 88.4 %, respectively, with a positive predictive value (PPV) of 71.5 % and a negative predictive value (NPV) of 95.1 %. Separate analysis for BALF and extrapulmonary samples showed varied sensitivity and specificity (Table 4). The highest diagnostic efficacy was observed for specimens of pericardial fluid.

Diagnostic efficacy of Xpert MTB/RIF for detection of RIF resistance

Table 5 compares the results of the Xpert MTB/RIF assay with the gold standard DST for detection of RIF resistance. The DST was done for 88 specimens that were culture-positive and resistance to RIF was observed in 24 (27.27 %) of these. On the other hand, the Xpert MTB/RIF assay detected RIF resistance in 28 (10.5 %) specimens out of 266. There were two cases where the Xpert MTB/RIF detected RIF resistance but DST showed no resistance to RIF. The Xpert MTB/RIF assay also detected resistance to RIF in two culture-negative specimens for which DST was not performed. In the present study the Xpert MTB/RIF assay showed 100 % sensitivity and NPV, whereas specificity was 96.9 % and PPV was 92.3 % for detection of RIF resistance when used against DST as a reference (Table 5).

DISCUSSION

TB is a major public health issue in developing countries where increases in MDR- and extensively drug-resistant (XDR)-TB strains have been reported [18, 19]. Delay in timely diagnosis of TB and drug-resistant TB (DR-TB) due to non-availability of rapid, more sensitive and specific techniques is a major concern in these countries. The problem can be intensified for countries such as Pakistan where the burden of extrapulmonary and smear-negative pulmonary TB is also high. The accurate and timely identification and rapid detection of DR-TB is of paramount importance in its prevention, treatment and control.
Molecular techniques have significantly improved the diagnosis of TB. They are rapid and highly sensitive [20]. Many studies have assessed the PCR assay using a number of different MTB targets for diagnosis [21, 22]. In the present study, we studied the clinical usefulness of Xpert MTB/RIF tests for the rapid diagnosis of TB and RIF resistance in both pulmonary and extrapulmonary TB suspects. Our pulmonary TB suspects were those who could not produce sufficient/effective sputum to enable sputum smears, and therefore we used BALF specimens. This is in contrast to a previous study in the region where sputum specimens were used [23, 24]. We also studied 168 extrapulmonary specimens of lymph node pus, CSF, and ascitic and pericardial fluid for MTB detection. The large number of specimens from unclassical TB sites adds to the significance of diagnostic use of the Xpert MTB/RIF assay for extrapulmonary cases of the disease.

Our study was carried out in the largest PMDT unit in KPK, Pakistan. This region is highly endemic for both TB and MDR-TB in the country. People from Afghanistan and conflict-prone tribal areas of Pakistan also frequently visit this treatment centre. This further signifies the increased external validity of our findings.

In the present study, the overall sensitivity and specificity of the Xpert MTB/RIF assay for diagnosis of TB was 86 and 88.4 %, respectively, with PPV 71.5 % and NPV 95.1 %. Sensitivity was 86 % in the current study, which is comparable to other published studies [25–31]; however, evidence exists for even higher sensitivity [23, 32, 33]. Specificity was 88.4 %, which is in line with the available evidence (89–99 %) [23, 26–28, 31–33]. The Xpert MTB/RIF assay has relatively higher sensitivity (98–100 %) and specificity (99–

<table>
<thead>
<tr>
<th>Culture results of specimens</th>
<th>Xpert MTB/RIF</th>
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<tbody>
<tr>
<td></td>
<td>MTB not detected (%)</td>
</tr>
<tr>
<td>BALF</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Contaminated</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Contaminated</td>
</tr>
<tr>
<td>CSF</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Pus (lymph nodes)</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Contaminated</td>
</tr>
<tr>
<td>Pericardial fluid</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Contaminated</td>
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<tr>
<td>Total</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Contaminated</td>
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</tbody>
</table>

Table 4. Diagnostic efficacy of the Xpert assay with culture method as the reference standard

<table>
<thead>
<tr>
<th>DST</th>
<th>RRD not detected (%)</th>
<th>RRD detected (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>62 (96.9)</td>
<td>2 (3.1)</td>
<td>100</td>
<td>96.9</td>
<td>92.3</td>
<td>100</td>
</tr>
<tr>
<td>RIF-resistant</td>
<td>0</td>
<td>24 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DST not applied</td>
<td>176 (98.9)</td>
<td>2 (1.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>238 (89.5)</td>
<td>28 (10.5)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

RRD, Rifampicin resistant detected.

Table 5. Comparison of the Xpert assay with drug susceptibility results for detection of RIF resistance
100%) in smear-positive pulmonary TB [7, 34–37] as compared to extrapulmonary specimens, where sensitivity is 37% in smear-negative and 100% in smear-positive specimens [34].

We observed high sensitivity (80%) of the assay for pulmonary TB (using BALF), which is comparable to sputum smear-negative pulmonary cases reported earlier [23]. However, specificity was low when compared to results reported from sputum samples in another study [23]. We found high sensitivity and specificity for all extrapulmonary specimens except for CSF (50%). Vadwai et al. also reported a relatively lower sensitivity (29%) of the Xpert MTB/RIF test for CSF specimens [38]. However, other studies have reported a higher sensitivity as compared to our findings [27, 28]. This lower sensitivity highlights the need for careful interpretation of the assay results for detection of neurological TB.

In our study, the Xpert MTB/RIF assay detected MTB in 80% of BALF specimens that were also culture positive and 95.83% of extrapulmonary culture-positive specimens. The Xpert MTB/RIF assay detected MTB in 22 cases, of which 18 were culture negative while in the remaining four cases the culture was contaminated. Contaminations of culture were due to endogenous bacteria resistant to the decontamination procedure. Furthermore, 6.8% (18/266) of culture-negative and Xpert MTB/RIF-positive cases might be due to the paucibacillary nature of extrapulmonary specimens with an affinity to MTB to form clusters, leading to an irregular distribution of bacteria and loss of viable bacteria during NALC-NaOH processing [28, 38, 39]. In the Xpert MTB/RIF assay, the sample reagent has a better liquefaction and homogenization efficacy than NALC-NaOH processing [28, 38, 39].

The sensitivity and specificity of the Xpert MTB/RIF assay for detecting RIF resistance is reported to be 94.4–100 and 98.3–100%, respectively, in previous studies [35, 36, 40]. Our study validates these findings, with 100% sensitivity and 96.9% specificity for detection of RIF resistance. We observed resistance to RIF in two culture-negative specimens using the Xpert MTB/RIF assay. The nature of the false-positive results would have been confirmed with rpoB sequencing. However, we were not able to sequence the rpoB gene in our study. Another limitation is that we did not have information on time to treatment, duration of hospitalization and other contact subjects.

In our study, 72 (72%) specimens were susceptible to RIF while 28 (28%) were resistant as per the Xpert MTB/RIF assay. We found relatively more cases of RIF resistance as compared to previous reports from Pakistan, China and Nicaragua [24, 41–43], but lower resistance than reported from Brazil (44.3%) by Luiz et al. [44]. Possible reasons for these differences could be sample size, methodology for selection of suspected DR-TB patients and geographical location. We observed RIF resistance in 25% of previously treated patients and in 3% of newly diagnosed patients, which is comparable to previous studies [45–49]. This highlights the need for use of the Xpert MTB/RIF assay for all previously treated cases for rapid detection of drug resistance. In the present study, the Xpert MTB/RIF assay showed 100% sensitivity and NPV, whereas specificity was 96.9 and PPV was 92.3% for RIF resistance using DST as a reference.

**Conclusion**

The Xpert MTB/RIF assay is a rapid and simple technique for detection of MTB and RIF resistance. This assay not only yields good sensitivity and specificity for the diagnosis of TB but also for detection of MDR-TB in BALF and in extrapulmonary TB. We recommend initiation of treatment for TB based on the findings of the Xpert MTB/RIF assay in Pakistan.


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