Identification of Mycobacterium tuberculosis and Rifampin Resistance in Pulmonary and Extra-pulmonary Clinical Specimens Using the Gene Xpert MTB/RIF Assay

ABSTRACT

Background and objectives: Tuberculosis (TB) is a serious public health problem and a significant diagnostic and therapeutic challenge worldwide. Molecular diagnostic techniques are crucial parts of the World Health Organization’s new tuberculosis control strategy. This study aims to identify Mycobacterium tuberculosis and rifampin resistance in pulmonary and extra-pulmonary clinical specimens using the Gene Xpert MTB/RIF assay.

Methods: The study was carried out on 220 specimens from pulmonary and extra-pulmonary TB patients that were sent to the Kavosh Laboratory in Gorgan (Iran) during 2018-20. The Gene Xpert MTB / Rif method was applied to detect M. tuberculosis and rifampin resistance.

Results: Of 220 specimens, 15 (6.81%) were found to be positive, four (26.6%) of which were related to pulmonary and 11 (73.3%) to extra-pulmonary specimens. None of the positive samples was resistant to rifampin according to assay.

Conclusion: Our findings demonstrate that the Gene Xpert MTB/RIF is able to accurately detect M. tuberculosis in pulmonary and extra-pulmonary specimens. The accurate and early diagnosis of TB infection allows timely therapeutic intervention, which is beneficial not only for the patient but also for possible contacts.

Keywords: Mycobacterium tuberculosis, Tuberculosis Multidrug-Resistant, Rifampin.
INTRODUCTION
Tuberculosis (TB) is one of the oldest known human diseases (1), which is caused by Mycobacterium tuberculosis complex (MTBC) (2). At least a quarter of the world’s population are currently infected with active or latent TB (1), and it is one of the leading causes of death worldwide (1, 3). According to the World Health Organization (WHO) report in 2018, more than 10 million new infections and 1.2 million deaths from TB occur annually, and an estimated 3.6 million cases remain undiagnosed each year (3-5). The advances in economic and public health status and the discovery of antibiotics have led to a reduction in the incidence of TB in industrialized countries in the early twentieth century. However, the emergence of antibiotic-resistant strains and the re-emergence of TB cases due to the HIV epidemic in the 1980s have once again made the disease an important public health issue (1).

Active TB often presents as a lung infection consisting of a prolonged cough that lasts for several weeks and bloody sputum. Other common symptoms include chills, fever, weakness, severe weight loss and night sweats. Latent TB is generally asymptomatic and patients may never notice that they are infected unless reactivation occurs (1,6). M. tuberculosis is responsible for 15% of extra-pulmonary infections, which may or may not be associated with pulmonary symptoms (5). Cervical lymphadenitis is one of the most common forms of extra-pulmonary TB infection. The second most common form of extra-pulmonary TB is pleural involvement, which causes an increase in pleural fluid between the lungs and the surrounding membrane. Gastrointestinal TB is another common form of extra-pulmonary infection that is caused by Mycobacterium bovis due to the consumption of contaminated raw milk. A more dangerous and less common form of extra-pulmonary TB is central nervous system (CNS) infection, which can occur in the form of tuberculous meningitis, encephalitis and abscess that is caused by the spread of bacteria throughout the bloodstream. This often leads to the formation of numerous small lesions known as miliary TB that can be formed in any body tissue but most frequently affect the lungs, liver, spleen, bone marrow and kidneys (7). Although TB is a preventable and treatable disease, the rising prevalence of multidrug resistant (MDR) and extensively drug resistant strains has created multiple challenges in the treatment of this disease (8). More than 400,000 cases of MDR TB occur annually (9, 10). The gold standard method of TB diagnosis is culture, which is hard and time consuming as it can take up to eight weeks to confirm the diagnosis. Although microscopic smear is a rapid and easy way to detect M. tuberculosis, it is insensitive and requires several sputum samples. Genotypic methods have significant advantages over other methods since they are faster and safer (3). The GeneXpert MTB/RIF is currently the only molecular test recommended by the WHO for the rapid diagnosis of TB. It can detect both the presence of the MTC genome in clinical specimens and the presence of genomic sequences of the main mutations responsible for rifampicin resistance (rpoB gene mutation). The time limit for reporting the result is two hours. Genotype MTBDRplus is a multiplex DNA amplification test coupled with hybridization on strips for routine identification of mycobacteria and detection of genomic sequences of anti-TB drug resistance. The result can be reported within a few hours and allows MTC and rifampicin and isoniazid resistance status to be detected in a single test (11). It performs well at diagnosing extra-pulmonary infections using lymph node, cerebrospinal fluid (CSF) and body fluids specimens (3). The aim of this study was to identify M. tuberculosis and rifampin resistance in pulmonary and extra-pulmonary clinical specimens using the Gene Xpert MTB/RIF assay.

MATERIALS AND METHODS
The study was performed on 220 clinical specimen from pulmonary and extra-pulmonary TB suspects that were sent to the Kavosh Laboratory (Gorgan, Iran) during 2018-20. M. tuberculosis and rifampin resistance were evaluated using the GeneXpert MTB/RIF test (Cepheid Sunnyvale, CA, United States). Clinical specimens included 14 pulmonary samples (sputum, bronchial aspirations and bronchoalveolar lavage) and 206 extra-pulmonary samples (biological body fluids and tissue samples). For pulmonary samples, the samples were centrifuged at 3000×g for 20
minutes. The supernatant was discarded and the precipitate was mixed with the kit solution in a ratio of 1:2 until reaching a total volume of at least 0.5 ml. The mixture was vortexed and left for 15 min at room temperature. For biological fluids, if the sample volume was more than 0.5 ml, the kit solution was added to the sample to reach the final volume of 2 ml. The mixture was vortexed and left for 15 min at room temperature. Tissue samples were first grinded with 2 ml of phosphate buffer saline using a tissue grinder. After completely homogenization, 0.7 ml of the solution were poured into a sterile falcon tube and 1.3 ml of the kit solution were added to reach a final volume of 2 ml. The mixture was vortexed and left for 15 min at room temperature. Next, 2 ml of the prepared solutions were transferred to the Xpert MTB/RIF cartridge and the automated steps of the procedure started immediately. Absence of a mutation in the rpoB gene indicated lack of rifampin resistance. Statistical analysis of data was performed with SPSS (version 18.0). The chi-square test was used to evaluate the relationship of M. tuberculosis frequency with demographic findings.

RESULTS
In this study, 220 clinical samples from 95 females (43.1%) and 125 males (56.8%) were included. Age of the patients was ranging from 8 months to 88 years. Table 2 shows the distribution of pulmonary and extra-pulmonary samples collected from TB suspects.

Table 1- Method of identification of M. tuberculosis using the GeneXpert MTB/RIF test

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<th>Ct range</th>
<th>High</th>
<th>Medium</th>
<th>Low</th>
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<td>&lt;16</td>
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<td>16-22</td>
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Table 2- Distribution of pulmonary and extra-pulmonary samples collected from TB suspects.

<table>
<thead>
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<th>Total number of specimens= 220</th>
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<tr>
<td>Pulmonary samples</td>
</tr>
<tr>
<td>n=14 (6.3%)</td>
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<tr>
<td>Sputum (n=3)</td>
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<tr>
<td>Bronchial aspirations (n=8)</td>
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<td>Bronchoalveolar lavage (n=3)</td>
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Of 220 samples, 15 samples (6.81%) were found to be positive in the GeneXpert MTB/RIF test. Moreover, four (28.5%) pulmonary, nine (4.8%) biological fluid and two tissue (9%) samples were positive for MTB using the Gene Xpert MTB/RIF. The highest frequency of TB positive cases was observed in body fluid samples, particularly CSF specimens (n=5).

Among 15 positive samples, six (40%) were taken from females and nine (60%) were taken from males. The bacterial concentration was high in three, moderate in seven, low in one and very low in four samples. No resistance to rifampin was observed in the 15 MTB positive samples.

DISCUSSION
As mentioned earlier, TB is one of the leading causes of death worldwide (1,3), and drug-resistant TB is one of the most serious health issues worldwide (12). The main reason for the increase in the prevalence of drug-resistant M. tuberculosis strains is the lack of access to appropriate means of early detection, which is mainly due to its high cost (13). Early diagnosis of TB is essential for initiating an effective treatment regimen and preventing its transmission in the community (14). Recent research has focused on molecular detection of bacteria from clinical specimens with acceptable turnaround
time (15). In October 2013, the WHO published a new guideline for using the GeneXpert MTB/RIF as a new generation of molecular automated operating systems for the diagnosis of pulmonary, pediatric, extra-pulmonary and rifampin-resistant TB (3,16). We analyzed 220 clinical samples using the GeneXpert MTB/RIF test. Of these samples, 15 (6.81%) were found to be positive for MTB. Among these cases, four (26.6%) were from pulmonary and 11 (73.3%) were from extra-pulmonary samples. The highest number of positive cases was related to body fluid samples. Our findings indicate that the GeneXpert MTB/RIF test performs well in detecting extra-pulmonary TB from different samples. In line with this finding, Habous et al. found that the GeneXpert MTB/RIF assay has a high sensitivity (82.69%) and specificity (100%) for detection of extra-pulmonary TB in non-respiratory samples when compared with culture (3). A study by Mechal et al. also showed that the GeneXpert MTB/RIF test has a high sensitivity (78.8%) and specificity (90.3%) for the diagnosis of extra-pulmonary TB samples when compared to microscopic examination (11). Therefore, introducing the GeneXpert MTB/RIF assay in the early diagnostic workflow can improve detection accuracy and shorten turnaround time. In addition, the earlier diagnosis of MTB and detection of drug resistance ultimately improve treatment outcome and survival.

CONCLUSION
The findings of this study confirm the GeneXpert MTB/RIF as a test of choice for the diagnosis of extra-pulmonary TB. The test allows accurate and early diagnosis of TB infection allowing timely therapeutic intervention, which is beneficial not only for the patient but also for possible contacts.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest regarding publication of this article.

REFERENCES


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