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Brief Original Article

Comparison of GeneXpert MTB/RIF and conventional methods for the diagnosis of tuberculosis in Kosovo

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Abstract

Introduction: Tuberculosis (TB) is a major public health problem worldwide, with the highest mortality occurring in developing countries. The burden of TB in Kosovo is among the highest in Europe. The aim of this study was to compare Cepheid GeneXpert MTB/RIF assay for direct detection of *Mycobacterium tuberculosis* complex (MTBC) and rifampin (RIF) resistance with conventional methods.

Methodology: A cross-sectional design to evaluate diagnostic tests was carried out at the Department of Microbiology, National Institute of Public Health of Kosovo and Lung Clinic, from January to June 2014. The detection of MTBC and RIF resistance using the Xpert MTB/RIF assay was assessed in 116 specimens received from 110 patients suspected of having TB and compared with conventional smear microscopy and culture methods.

Results: Fifty-eight patients (52.7%) were male, and the mean age was 48.6 ± 18.1 years. Twenty-nine patients (26.4%) had underlying lung diseases. Of the 116 specimens investigated, 28 (24.1%) were MTBC-positive by culture, while 34 (29.3%) were positive by Xpert assay. Two samples showed false-negative Xpert results. Compared with culture, the Xpert assay achieved 82.3% (95% CI: 65.5%–93.2%) sensitivity, and 97.6% (95% CI: 91.5%–99.7%) specificity. GeneXpert could detect 11.7% and 50% additional positive cases as compared to Lowenstein-Jensen culture and smear microscopy, respectively. Three cases with resistance to rifampin were detected from clinical isolates.

Conclusions: The GeneXpert MTB/RIF assay is a helpful tool for rapid diagnosis and prompt treatment of TB.

Key words: tuberculosis; diagnosis; molecular.

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Introduction

Tuberculosis (TB) remains a major public health problem worldwide. It is the second-highest cause of death among communicable diseases. According to the World Health Organization (WHO), in 2013, 9 million people fell ill with TB and 1.5 million died from TB. Over 95% of TB deaths occur in low- and middle-income countries, and TB is among the top five causes of death for women 15 to 44 years of age [1]. TB treatment and control have been severely compromised in recent years due to the increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB [2].

Kosovo, with a population approaching 2 million, is located in the southeast of Europe. Healthcare in Kosovo underwent important reforms in the last decade, facing immense difficulties and obstacles, of which the most important were lack of political commitment and scarce resources. No health insurance system has been established yet, which represents a key obstacle to any effort to improve the healthcare levels in all sectors [3]. Since 1999, TB has re-emerged as a public health problem in Kosovo. The burden of TB is among the highest in Europe, with a notification rate of 46 per 100,000 population in 2012. TB control has been relatively successful: the number of registered cases decreased from 1,443 cases in 2002 (69 per 100,000 population) to 834 in 2014 (46 per 100,000), an annual median decline of 3.5%. During the last year, there were eight cases with multidrug-resistant TB in Kosovo. There has been a decreasing trend in notified cases until 2006, when the number of cases remained more or less stable [4,5].

Microscopy, culture, and drug susceptibility testing (DST) are standard methods used in laboratory diagnosis of TB across the world, but they are time-consuming processes, taking up to eight weeks for diagnosis. Doctors in the developing world often rely only on chest X-rays without referring patients for sputum smears; confirmation by culture is not done routinely. Moreover, diagnosis of extrapulmonary TB is difficult to establish due to the low number of bacteria in clinical specimens. Rapid and accurate diagnosis of pulmonary and extrapulmonary TB is still a great

challenge in developing countries due to limited resources and a lack of laboratory expertise [6]. The emergence and spread of drug resistance represents a challenge for healthcare systems and jeopardizes TB infection control efforts [7].

Therefore, new developments in molecular diagnostics have been introduced into practice, playing a pivotal role in early diagnosis of and prompt response to TB. Advances in molecular diagnostics, drugs, and vaccines have improved chances for better TB control at a global level [8]. Several molecular methods have been developed during the last decade for TB diagnosis and rapid detection of TB resistance, including line probe assays GenoType MTBDRplus (Hain Lifescience GmbH, Nehren, Germany) and INNO LIPA Rif.TB (Innogenetics, Ghent, Belgium), and real-time polymerase chain reaction (PCR) GeneXpert MTB/RIF (Cepheid, Sunnyvale, USA) [9].

The GeneXpert MTB/RIF assay is a hemi-nested real-time PCR assay for the diagnosis of TB as well as rapid detection of rifampin (RIF) resistance in clinical specimens within two hours [10].

The aim of this study was to evaluate Cepheid GeneXpert MTB/RIF assay for direct detection of *M. tuberculosis* and RIF resistance and compare it with conventional culture methods.

Methodology

This descriptive and prospective study was conducted during the first part of the year 2014 in the TB Laboratories of the Lung Clinic at University Clinical Center of Kosovo and in the Department of Microbiology within the National Institute of Public Health of Kosovo, Prishtina, Kosovo. The specimens collected were from patients with suspected *M. tuberculosis* infection on the basis of clinical criteria. A total of 116 samples were enrolled in the study. Among respiratory specimens, 92 samples were sputum, 2 were bronchoalveolar lavage, and 11 were thoracentesis fluid. Non-respiratory samples included 7 cerebrospinal fluid and 4 urine samples.

Both respiratory and non-respiratory specimens were processed by the standard N-acetyl-L-cysteine and sodium hydroxide method. Freshly prepared Mycoprep NALC-NaOH solution (Becton Dickinson, Sparks, USA) was added to the specimens at equal volume, mixed on vortex, and left for 15 minutes for digestion at room temperature. A double amount (twice the amount of mixture) of sterile phosphate buffer (pH 6.8) (Becton Dickinson, Sparks, USA) was then added to the mixture and centrifuged for 20 minutes at 3,000 rpm. The supernatant was removed and the sediment was dissolved in 2.5 mL of sterile phosphate buffer for further study.

Lowenstein-Jensen (LJ) medium (Liofilchem Diagnostici, Roseto d'Abruzzi, Italy) was used to inoculate with 0.5 mL of dissolved specimen solution. The inoculated LJ medium was then incubated at 37°C for eight weeks and examined weekly, while 0.5 mL of the specimen solution was also added to liquid medium in Mycobacterium Growth Indicator Tubes (MGIT) (Becton Dickinson, Sparks, USA). The MGIT tubes were then incubated in an automated MGIT 960 system (Becton Dickinson, Sparks, USA) at 37°C for six weeks. The remaining deposit was used for PCR investigation using the Xpert MTB/RIF assay (Cepheid, Maurens-Scopont, France). One milliliter of the remaining deposit of clinical sample was transferred to a screw-capped tube containing 2 mL of sample reagent at a ratio of 1:2; this reagent inactivates the sample with NaOH and isopropanol. The mixture was then incubated for 15 minutes at room temperature and mixed every five minutes until liquefied with no visible clumps. The mixture was transferred into the Xpert MTB/RIF cartridge using the sterile pipette provided until the meniscus was above the minimum mark. The Xpert MTB/RIF cartridge includes an internal control for sample processing (DNA extraction and for PCR presence inhibitors). The inoculated cartridge was placed into the GeneXpert instrument (GX). Results were available in less than two hours and interpreted by the GX system automatically as follows: positive or negative results were related to the presence or absence of MTB DNA, while false results were due to the presence of PCR inhibitors.

Sterile samples were processed directly without contamination and digested followed by centrifugation at 3,000 rpm for 20 minutes. The supernatant was discarded and the sediment was resuspended with 2.5 mL of sterile phosphate buffer and used for acid-fast bacilli (AFB) microscopy, culture, and Xpert PCR as mentioned above. Smear examination for presence of AFB was carried out routinely for all specimens. Smears were prepared, fixed, and stained with Ziehl-Neelsen stain (Liofilchem Diagnostici, Roseto d'Abruzzi, Italy) and then visualized under an Olympus fluorescence microscope (Olympus, New York, USA) at 400× magnification.

Positive cultures of *Mycobacterium* isolate were investigated by smear examination, pigment production, biochemical tests such as nitrate reduction, and niacin accumulation tests (Becton Dickinson, Sparks, USA).

After growth of the cultures and species identification and, in cases in which MTBC strains were identified, DST was performed on LJ media using the proportional method [11].

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Xpert assay and sputum acid-fast smear were compared to TB culture as a reference standard using contingency two-by-two tables. P values less than 0.05 were taken as statistically significant. All statistical analyses were performed using MedCalc statistical software version 16.2 [12].

The study was conducted after approval by the research ethics review committee of the University of Prishtina, Kosovo.

Results

A total of 116 specimens (105 respiratory and 11 non-respiratory) from 110 patients with suspected TB infection were assayed for TB detection by Xpert PCR and conventional methods. Fifty-eight patients (52.7%) were male, and the mean age was 48.6 ± 18.1 years. Twenty-nine patients (26.4%) had underlying lung diseases. There were no patients with HIV infection.

A confirmed positive culture of MTBC was used as a reference standard. Out of 116 specimens, 28 (24.1%) were positive for *M. tuberculosis* by culture (MGIT and/or LJ media), 34 (29.3%) were found positive with Xpert MTB/RIF, while 17 (14.6%) were positive with the Ziehl-Neelsen staining method. Comparison between molecular and conventional methods is presented in Table 1.

One positive smear for AFB was negative by Xpert assay because this was non-tuberculous *Mycobacterium*. Two samples showed false-negative Xpert results compared to the reference method.

Table 1b shows the two-by-two contingency table comparing sputum acid-fast smear and sputum culture. Using sputum culture as a reference standard, the overall sensitivity, specificity, PPV, and NPV for sputum acid-fast smear were 53.3%, 98.8%, 94.1%, and 85.8%, respectively.

The sensitivity, specificity, PPV, and NPV and their corresponding 95% CIs are shown in Table 2. The sensitivity, specificity, PPV and NPV of Xpert MTB/RIF test were estimated as 93.3%, 93.0%, 82.3%, and 97.5%, respectively. Xpert assay had statistically significant higher sensitivity than the sputum acid-fast smear ($p < 0.001$).

Three cases with resistance to rifampin were detected from clinical isolates.

GeneXpert could detect 11.7% and 50% additional positive cases as compared with LJ culture and smear microscopy, respectively.

Regarding the sex, age, and underlying lung disease within the studied cohort, there was no significant difference in correlation with *M. tuberculosis* detection methods.

Discussion

Conventional methods for detection of MTBC in clinical specimens have low sensitivity [13]. Molecular techniques, including the Cepheid GeneXpert system, have changed the field of TB with rapid diagnosis

Table 1. Two-by-two contingency tables comparing Xpert assay (a) and sputum acid-fast smear (b) with the reference standard.

a)	**Positive Xpert assay**	**Negative Xpert assay**	**Total**
Culture grew *M. tuberculosis*	28	2	30
Culture grew NTM or no growth	6	80	86
Total	34	82	116
b)	**Positive smear**	**Negative smear**	**Total**
Culture grew *M. tuberculosis*	16	14	30
Culture grew NTM or no growth	1	85	86
Total	**17**	**99**	**116**

NTM: non-tuberculous mycobacteria.

Table 2. Sensitivity, specificity, positive predictive value, and negative predictive value of Xpert assay and sputum acid-fast smear comparing to sputum culture.

	Xpert assay	**95% confidence interval**	**Acid-fast smear**	**95% confidence interval**
Sensitivity	82.3%	65.5%–93.2%	94.1%	71.3%–99.8%
Specificity	97.6%	91.5%–99.7%	85.7%	77.4%–92.1%
Positive predictive value	93.3%	77.9%–99.1%	53.3%	34.3%–71.6%
Negative predictive value	93.0%	85.4%–97.4%	98.8%	93.7%–99.9%

combined with high sensitivity and specificity results. In December 2010, the WHO endorsed the Xpert MTB/RIF assay for the rapid diagnosis of TB and MDR-TB [14].

Many studies have assessed the molecular techniques for detection of MTBC, and the results indicated that PCR is a useful approach for rapid diagnosis of TB from clinical specimens. However, there are limitations to these techniques related to extraction procedures and the presence of PCR inhibitors in some of the clinical specimens.

A systematic review by Steingart *et al*. of 27 studies showed that Xpert assay of respiratory specimens had a pooled sensitivity of 89% (95% CI: 85%–92%) and specificity of 99% (95% CI: 98%–99%) in the diagnosis of pulmonary TB [15]. There were different results from different studies. The difference in sensitivity of the Xpert assay on detection of *M. tuberculosis* among studies could be explained by differences in inclusion criteria and techniques used to obtain sputum specimens. Our results are compatible with those of these previous studies; the sensitivity of Xpert assay to detect *M. tuberculosis* was 96.4% in acid-fast positive sputum specimens. Results similar to those in our study were obtained in Saudi Arabia, Pakistan, Thailand, and Turkey [16-19]. Our study and other studies all confirm that positive acid-fast smear correlated well with Xpert assay and TB culture.

Invalid and error results from Xpert assay occurred in 3/116 specimens (2.6%), whereas contaminated culture occurred in 5/116 specimens (4.3%). False-negative Xpert PCR results were detected in two specimens. The possible explanation is the presence of PCR inhibitors or insufficient nucleic acid material in these specimens.

The main limitations of our study were the relatively small number of samples and insufficient number of positive samples to evaluate Xpert performance in detecting rifampin resistance.

In Kosovo, TB diagnosis is based on chest X-ray or clinical considerations, without bacteriological confirmation. Even when sputum smears are requested, only a small proportion of them are confirmed by culture, mostly on solid (slow) media. Rapid molecular tests have recently been introduced in Kosovo and are now used for presumptive TB cases. Xpert MTB/RIF is only used at the request of a clinician. Priority was given to extrapulmonary samples and samples from children with presumptive TB.

The high cost of the Xpert assay could be an important barrier in a TB control program. The limited number of tests was due to economic problems: in Kosovo, a single Xpert MTB/RIF test costs 90 EUR, which is unsustainable. The internationally negotiated price is about 10 EUR per test.

Conclusions

This study demonstrated good sensitivity and specificity of Xpert assay in detecting *M. tuberculosis*. This diagnostic test is helpful for rapid diagnosis and prompt treatment of TB, particularly in patients who had a negative sputum acid-fast smear.

Because the smear microscopy (sputum AFB) test had some problems in HIV-positive patients, children, and patients with low bacterial load, the use of GeneXpert MTB, which has a high sensitivity and specificity, is important in diagnosis of TB disease. The GeneXpert MTB assay should be evaluated in HIV-infected patients.

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