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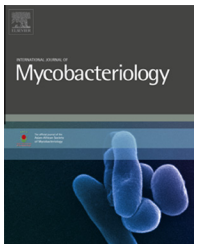

Short Communication

Evaluation of Xpert MTB/RIF assay for rapid molecular diagnosis of tuberculosis in a two-year period in Croatia

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ABSTRACT

Mycobacterium tuberculosis remains a major global health problem and is currently killing 1.5 million people every year. One of the most important steps in tuberculosis control is the rapid and accurate laboratory diagnosis. The Xpert MTB/RIF assay is a novel molecular, easy-to-use assay, which can lead to tuberculosis identification in less than 2 h. In this study, the Xpert MTB/RIF assay performance for rapid diagnosis of tuberculosis was evaluated in comparison with conventional culture methods; 361 pulmonary and extrapulmonary patient samples were collected between October 2010 and October 2012 and were analyzed at the National Reference laboratory for Mycobacteria, Zagreb, Croatia. For pulmonary samples the sensitivity and specificity were 86% and 100%, while for extrapulmonary samples the sensitivity and specificity were 75% and 99%, respectively. It was concluded that Xpert MTB/RIF assay has high sensitivity and specificity for both pulmonary and extrapulmonary specimens.

Introduction

Mycobacterium tuberculosis remains a major global health problem and is currently killing three persons every minute. Although the tuberculosis (TB) death rate dropped more than 40% between 1990 and 2011, more has to be done in order to reach the Stop TB partnership goal of zero tuberculosis deaths in this lifetime [1,2]. While waiting for a new and efficient TB vaccine to come in use, other key points in TB control must be addressed. One of the most significant steps in TB control is the fast and accurate disease diagnosis. Although culture is the gold standard for final disease determination, it is slow and actually out of reach in low-income countries. In the last couple of years there has been a major breakthrough in the diagnosis of TB owing to the discovery of fast molecular methods [3]. The Xpert MTB/RIF is a novel automated molecular assay recently endorsed by the World Health Organization for the early diagnosis of both *M. tuberculosis* infection and rifampin resistance [4]. The sample processing, nucleic acid extraction, amplification and detection of rifampicine resistance are performed in a single cartridge within 2 h. The assay velocity in detecting both *M. tuberculosis* and rifampicine resistance enable us to stop the spread of disease and give appropriate treatment considerably faster compared with conventional methods.

The researchers from the Cochrane Infectious Diseases Group, McGill University, and the Foundation for Innovative New Diagnostics (FIND) have recently analyzed data from 18 studies involving a total of 7816 people, in order to review the diagnostic accuracy of Xpert MTB/RIF assay [5]. The review

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shows that XpertMTB/RIF assay has a high sensitivity and specificity when used as an initial diagnostic test for TB and rifampicine resistance detection in patients suspected of having TB [5].

The Xpert MTB/RIF assay has been in use at the National Reference Laboratory (NRL) for Tuberculosis of the Croatian National Institute of Public Health (CNIPH) since October 2010. With 680 newly diagnosed patients in 2011, the incidence of tuberculosis in Croatia is 15/100,000 population [6]. The proportion of resistant strains among new isolates is 3%, while the proportion of multidrug-resistant (MDR) tuberculosis is below 1% [6]. Although the NRL performs a third-level laboratory service for the whole country, it is actually also involved in first- and second-level laboratory work for several counties.

The aim of the present study was to determine the performance of the Xpert MTB/RIF assay for the rapid diagnosis of tuberculosis and rifampicine resistance from pulmonary and extrapulmonary patient samples. The results obtained with the Xpert MTB/RIF assay were compared with conventional culture methods.

Material and methods

Clinical samples

All samples included in the study were collected between October 2010 and October 2012 at the National Reference laboratory for Mycobacteria, Zagreb, Croatia. Out of 361 total clinical samples collected, 120 were pulmonary and 241 were extrapulmonary; 309 were suspected tuberculosis patients. The 120 pulmonary samples included 51 bronchial aspirates, 24 bronchoalveolar lavages, 42 sputum and 3 gastric lavages. The 241 extrapulmonary samples included 50 urine, 46 cerebrospinal fluid (CSF), 58 tissue samples, 10 ascitic fluid, 11 blood samples, 17 pericardial fluid, 42 pleural fluid, 4 skin swabs and 3 stool specimens.

Non-sterile specimens were processed by the standard N-acetyl-L-cysteine and sodium hydroxide (NALC/NaOH) method and smears were prepared by the auramine acid-fast staining method. After decontamination, specimens were inoculated to both solid and liquid mediums.

MGIT 960

MGIT tubes were inoculated with 0.5 ml of the processed specimen. The tubes were incubated in the MGIT 960 instrument at 37 °C for up to 6 weeks. For tubes identified as positive, a smear of a sample from the tube was prepared for examination for acid-fast bacilli (AFB) by Ziehl–Neelsen stain. Further differentiation of mycobacteria was performed with molecular methods and biochemical tests (CM/AS/MTBC assay, Hain Lifescience, GmbH, Nehren, Germany).

Solid media

For each specimen, one Lowenstein–Jensen (LJ) slant and one Stonebrink slant were inoculated with 0.1 ml suspension and incubated at 37 °C. Bacterial colonies were investigated by AFB smear and were further investigated by molecular methods and biochemical tests.

DST

Drug susceptibility testing (DST) for rifampicine was performed for all culture-positive samples with the proportion method on LJ solid medium as previously described (standard critical concentration of 40 μg/ml).

Xpert MTB/RIF procedure

Xpert MTB/RIF assay was performed and results were interpreted according to the manufacturer's instructions [7]. Briefly, sample reagent was added in a 3:1 ratio to 0.5 ml of decontaminated specimen. The closed tube was manually agitated twice during a 15-min incubation period at room temperature before 2 ml of the reagent-sample mixture was transferred to the Xpert test cartridge. Cartridges were inserted into the Xpert device, and the automatically generated results were read after 90 min.

Results

Patients and conventional culture

A total of 361 clinical samples originating from 309 suspected tuberculosis patients were analyzed, of which 120 were pulmonary and 241 were extrapulmonary.

Of the 120 pulmonary samples, 7 were culture-positive for *M. tuberculosis* and 2 were culture-positive for *Mycobacterium intracellulare*. The rest of the 111 samples were culture-negative (Table 1).

Of the 241 extrapulmonary samples, 12 were culture-positive for *M. tuberculosis* complex (MTC), while 229 were culture-negative. From the 12 culture-positive samples, 9 were identified as *M. tuberculosis*, while 3 were identified as *Mycobacterium bovis* (Table 1).

Xpert MTB/RIF assay performance

Of the 7 culture-positive pulmonary samples, 6 were also positive withXpert MTB/RIF analysis (3 sputum, 2 bronchial aspirates and 1 bronchoalveolar lavage fluid). Three of the 7 culture-positive samples were also smear-positive (2 sputum and 1 bronchial aspirate), and all were identified as *M. tuberculosis* with Xpert MTB/RIF assay.

Of the 12 culture-positive extrapulmonary samples, 9 were also positive with Xpert MTB/RIF analysis (5 tissue samples, 2 skin swabs, 1 peritoneal fluid and 1 CSF). One tissue sample and both skin samples, which were found culture-positive and Xpert MTB/RIF positive, were identified as *M. bovis*. All the culture-positive samples, except skin swabs, were smear-negative. There were also two CSF samples identified positive with Xpert MTB/RIF assay and negative with conventional culture methods.

According to the conventional culture results, the sensitivity of Xpert MTB/RIF analysis for all samples was 79%, and the specificity was 99% (Table 2).

For pulmonary samples, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 86%, 100%, 100% and 99%, respectively. The sensitivities

Table 1 – The specimens analyzed and smear and culture results.

Specimen type	Smear +	Smear −	Culture (MTC)+	Culture (MTC)−	*M. intracellulare*
Pulmonary (n = 120)					
Bronchial aspirates (*n* = 51)	1	50	2	48	1
Sputum (*n* = 42)	2	40	4	37	1
BAL (*n* = 24)	0	24	1	23	0
Gastric lavage (*n* = 3)	0	3	0	3	0
Extrapulmonary (n = 241)					
Urine (*n* = 50)	nd[a]	nd[a]	0	50	0
CSL (*n* = 46)	0	46	1	45	0
Pleural fluid (*n* = 42)	0	42	1	41	0
Pericardial fluid (*n* = 17)	0	17	0	17	0
Blood (*n* = 11)	0	11	0	17	0
Tissue (*n* = 58)	0	58	5 (1*M.bovis*)	53	0
Ascitic fluid (*n* = 10)	0	10	3	7	0
Skin swab (*n* = 4)	2	2	2 (2*M.bovis*)	2	0
Stool (*n* = 3)	nd[a]	nd[a]	0	3	0

[a] Not done.

were 100% and 75% for smear-positive and smear-negative specimens, respectively.

For extrapulmonary samples, the sensitivity, specificity, PPV and NPV were 75%, 99%, 75% and 99%, respectively. The sensitivity for smear-positive samples was 100%, while for smear-negative specimens it was 70%.

The specificity and NPV for smear-positive pulmonary and extrapulmonary samples was not calculable, as there were no negative test results.

Susceptibility testing was performed for all culture-positive samples and all isolates were found to be susceptible to rifampicine with both conventional proportion method and XpertMTB/RIF testing. Moreover, the isolates tested with the conventional proportion method were found to be susceptible to all first-line antituberculotic drugs.

Discussion

One of the most important steps in tuberculosis control is the rapid and accurate laboratory diagnosis which leads to faster treatment and faster epidemiological control. In this study, the aim was to investigate the performance of Xpert MTB/RIF assay, a rapid molecular test, in the routine laboratory work at the National Reference Laboratory for Mycobacteria, Zagreb, Croatia. Previous studies have reported high sensitivity and specificity for Xpert MTB/RIF assay, especially in smear positive samples. In the present investigation, high sensitivity and specificity for smear-positive, both pulmonary and extrapulmonary specimens, was also detected. Lower sensitivity was observed in smear-negative pulmonary samples (75%), and especially in smear-negative extrapulmonary samples (70%). However, considering that the Xpert MTB/RIF test is finished in 3 h, its sensitivity is considerably higher than those of smear microscopy which in was 43% in this study in comparison with Xpert MTB/RIF which was 79%. Moreover, it would be interesting to asses the usefulness of this method on extrapulmonary samples where the smear is not carried out. In the routine analysis of urine or stool specimens for *M. tuberculosis* infection, one depends on culture results, which are time-consuming and actually out of reach for many field laboratories. Unfortunately, there was no culture-positive result from urine or stool samples in this study, but previous studies described high specificity of Xpert MTB/RIF in detecting *M. tuberculosis* in urine and stool specimens [8].

When analyzing pulmonary samples, Xpert MTB/RIF did not detect *M. tuberculosis* DNA in only 1 sputum sample out of 7 culture-positive samples. However, when looking at extrapulmonary samples, XpertMTB/RIF missed 3 *M. tuberculosis* infections, including 1 pleural fluid sample and 2 ascitic fluid samples. Low detection of *M. tuberculosis* in ascitic fluid

Table 2 – Sensitivity, specificity, and positive and negative predictive values of the GeneXpert MTB/RIF assay with culture method as gold standard.

Specimen type	Sensitivity (%)	Specificity (%)	PPV(%)	NPV(%)
All samples	79	99	83	99
Pulmonary (all)	86	100	100	99
Smear +	100	nc[a]	100	nc[a]
Smear −	75	100	100	97
Extrapulmonary (all)	75	99	75	99
Smear +	100	nc[a]	100	nc[a]
Smear −	70	99	70	99

[a] Not calculable.

samples was already observed by Zeka et al., which found that Xpert MTB/RIF missed 3 out of 4 positive ascitic fluid samples [9].

In this study, two CSF samples and one tissue sample were identified as positive using Xpert MTB/RIF assay and negative using conventional culture methods. One patient with a culture-negative/Xpert positive result from CSF was subsequently positive in further sample analyses on conventional culture and was diagnosed as tuberculosis infection. The other two culture-negative/Xpert positive patients were diagnosed as active tuberculosis and clinically recovered after antituberculotic treatment. These results indicate the possible value of Xpert MTB/RIF assay in detecting *M. tuberculosis* in samples of small bacillary load, especially CSF samples.

Drug susceptibility testing was performed for all culture-positive samples and all isolates were found to be susceptible to rifampicine with both conventional proportion method and Xpert MTB/RIF testing.

Conclusion

From this investigation, it can be concluded that Xpert MTB/RIF assay has high sensitivity and specificity for both pulmonary and extrapulmonary specimens. Moreover, it was found that this molecular assay is a very fast and useful tool for the rapid diagnosis of tuberculosis infection, especially in some extrapulmonary samples with low bacillary load.

REFERENCES

[1] S. Keshavjee, M. Harrington, G. Gonsalves, L. Chesire, P.E. Farmer, Time for zero deaths from tuberculosis, The Lancet 378 (2011) 1449–1450.
[2] WHO, Global Tuberculosis Report 2012, World Health Organization, Geneva, Switzerland, 2012.
[3] M.L. Wilson, Recent advances in the laboratory detection of *Mycobacterium tuberculosis* complex and drug resistance, Clin. Infect. Dis. 52 (2011) 1350–1355.
[4] C.C. Boehme, P. Nabeta, D. Hillemann, M.P. Nicol, S. Shenai, F. Krapp, et al, Rapid molecular detection of tuberculosis and rifampin resistance, N. Engl. J. Med. 363 (2010) 1005–1015.
[5] K.R. Steingart, H. Sohn, I. Schiller, L.A. Kloda, C.C. Boehme, M. Pai, et al., Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults, Cochrane Database of Systematic Reviews 2013, Issue 1, Art. No.: CD009593, doi: 10.1002/14651858.CD009593.pub2.
[6] Croatian Heath Service Yearbook 2011, Croatian National Institute of Public Health 2012, Zagreb, Croatia.
[7] D. Helb, M. Jones, E. Story, C. Boehme, E. Wallace, K. Ho, et al, Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology, J. Clin. Microbiol. 48 (2010) 229–237.
[8] D. Hillemann, S. Rüsch-Gerdes, C. Boehme, E. Richter, Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system, J. Clin. Microbiol. 49 (2011) 1202–1205.
[9] A.N. Zeka, S. Tasbakan, C. Cavusoglu, Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens, J. Clin. Microbiol. 49 (12) (2011) 4138–4141.