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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ARTICLE INFO

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 with Xpert 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