Original Article

Evaluation of rapid GeneXpert MTB/RIF method using DNA tissue specimens of vertebral bones in patients with suspected spondylitis TB

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ABSTRACT

Purpose: To detect *Mycobacterium tuberculosis* DNA and rifampicin resistance in vertebral bone tissue specimens from spondylitis TB suspects.

Methods and results: The rapid GeneXpert MTB/RIF and MGIT 960 liquid culture methods have been used in the specimens. Results from 70 suspects with spondylitis TB shown that 31.42% identified positive for spondylitis TB using culture method, while 88.57% shown positive results using rapid GeneXpert MTB/RIF method. The validity of GeneXpert MTB/RIF shown sensitivity value of 100%, specificity value of 16.6%, PPV of 35.48%, and NPV of 100%.

Conclusion: GeneXpert has a high sensitivity but low specificity value in this study.

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1. Background

Epidemiologically, tuberculosis rises as one of the deadliest diseases in the world. Indonesia ranked third among TB-high burden countries after India and China.¹ The data from World Health Organization estimates more than 6 million of the population are infected with tuberculosis and causing 1.5 million deaths worldwide.² Spondylitis tuberculosis is one of the type of TB infection that accounts for approximately 1–5% of all TB cases.³ Spondylitis TB is also known as Pott's disease, an infection of *Mycobacterium tuberculosis* that attacks the vertebral bone.^{4,5}

Spondylitis TB accounts for 50% of all tuberculosis in bones and joints. On developing countries, 60% of spondylitis TB reported attacking population under 20 years old, while it commonly strikes more elderly population in developed countries. Although it is not merely significant, this disease is more often discovered in male than female population.⁵ Spondylitis TB begins to spread by a hematogenic route from an organism, begin to grow in the anterior

aspects of vertebral bodies, close to the intervertebral disc. The inflammatory process will start to scrapes cortical parts of bone, and involve the nearest vertebral body from the inflammatory area.^{5–7} Healing in spondylitis TB can produce a partial or complete fusion of vertebral bodies. The possibility of an infection can be greater if the upper site of thoracic and lumbar aspects of the vertebra is lower than normal.^{5,6}

Diagnosis of spondylitis TB can be confirmed using a complete clinical evaluation, including the recent history of contacts with TB patient, epidemiological assessment, clinical symptoms, and neurological examination. Acid fast staining and microbiological culture shall be performed to confirm the infection of *M. tuberculosis* in suspected cases.⁸ Acid fast staining is the rapid screening method to detect *M.tb*, but is considered weak due to its low sensitivity rate. Meanwhile, microbiological culture is recognized for having a high sensitivity and specificity value but needed around 14 days to provide results.^{8,9} Currently, radiological examination is widely used as an early diagnostic approach for Spondylitis TB due to its ability to directly visualized the physical abnormalities in the vertebral bone. Several techniques that can be performed are X-ray, Computed Tomography Scan (CT scan), and Magnetic Resonance Imaging (MRI).^{5,10} Currently, GeneXpert MTB/RIF is one of the recent discovery in the fields of molecular diagnostic technique.

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GeneXpert MTB/RIF is one of the current systems that is widely explored to detect tuberculosis.¹¹ GeneXpert MTB/RIF using heminested real-time polymerase chain reaction (PCR) assay to multiply specific DNA sequences of *M. tuberculosis* from the rifampicin-resistant *rpoB* gene. Rifampicin resistance (RMP) from GeneXpert is discovered by determining the area of the *rpoB* gene that will be examined using molecular beacon as the probe.¹² This evaluation can be conducted automatically, including bacterial lysis, extraction and amplification of nucleic acid, and the detection of the amplicon. The system will be running on the GeneXpert platform (Cepheid, Sunnyvale, CA) by using disposable cartridge that contains a previously-determined reagent.¹³ GeneXpert provided as a simple method that works automatically, take two hours, and is not require an expert to perform this examination. Several reports described the success of GeneXpert on detecting every pulmonary TB cases among all suspects, including more than 90% of suspects with negative results in acid fast staining examination. It also had 97% sensitivity rate.^{11,14}

The Medical Research Council Committee for Research of Tuberculosis in the Tropics described that isoniazid and rifampicin must be regularly given during the period of treatment. This statement is parallel with a study conducted by Karaeminogullari et al.¹⁵ that cures spondylitis TB patient with rifampicin and isoniazid for nine months with satisfying results. Meanwhile, there were cases when some of anti-tuberculosis drugs, especially rifampicin and isoniazid, proved to be resistant. This phenomenon is known as multidrug-resistant TB (MDR-TB). Recently, the detection of rifampicin resistance as the sign of multidrug-resistant TB (MDR-TB) is already recommended by WHO.¹ Thus, GeneXpert MTB/RIF can be utilized to detect *M.tb* and rifampicin resistance, simultaneously.^{14,16,17}

2. Materials and methods

2.1. GeneXpert MTB/RIF

GeneXpert MTB/RIF test is conducted using a modified *Helb* method.¹⁸ A specimen of vertebral bone that contains blood, pus, and tissues from spinal surgery will be crushed and centrifuged for 10 min. Then, the supernatant of the samples will be added to a buffer solution with a comparison of 1:2. Sample and buffer will be vortex and incubated for 15 min at room temperature. Two milliliters of the results will be inserted into the cartridge. Cartridge will be included into the GeneXpert MTB/RIF device to be run for two hours. The results will be automatically read after two hours.

2.2. Decontamination

The tissue specimen of vertebral bones will be decontaminated using a standard method of N-acetyl-L-cysteine and sodium hydroxide (NALC/NaOH) with NaOH concentration of 1%.¹¹ After centrifugation, the pellet will be suspended into 1–1.5 ml of sterile buffer phosphate (pH 6.8). Then, samples will be centrifuged at a speed of $3000 \times g$ for 15 min. We will then remove the supernatant slowly and add 1–2 ml of buffer phosphate. The results of decontamination will be used for liquid culture on mycobacteria growth indicator tube (MGIT 960).

2.3. Culture in MGIT 960 liquid medium

500 μ l of the specimen from decontamination process will be inserted into MGIT 960 tube that has been added with 100 μ l PANTA (polymyxin amphotericin nalidixic acid trimethoprim azlocillin) AND 500 μ l OADC (Oleic Acid Albumin Dextrose Catalase). Then, it will be incubated at 37 °C temperature for

± 42 days. The reading process will be conducted using MGIT reader. If the devices fluorescent on the range of 2–14 (bright orange colored area), the interpretation of culture/media is positive. Contrary, if the fluorescence rise in the range of 9–11 (an orange colored area similar with negative control), the interpretation is negative. Growth in the culture can also be observed by the appearance of homogeneous turbidity, particle, or sediment inside the medium.

2.4. DST (drug-susceptible test) MGIT

DST test to the rifampicin will be performed using Bactec MGIT 960 (MGIT 960; Becton Dickinson Diagnostic System) method using the standard concentration of 1 μ g/ml. 500 μ l samples (positive culture) will be inserted into MGIT medium, and then added with OADC and PANTA. Then, it will be incubated at 37 °C temperature. Each of the samples will use two MGIT tubes (control and intervention with by adding 1 μ g/ml of rifampicin). Observation will be started on the third to twelfth day post inoculation.

3. Results

The detection of *M.tb* in tissue specimens of vertebral bones is conducted using GeneXpert MTB/RIF rapid method and MGIT liquid culture method. In this study, there are 62 samples (88.57%) among 70 individuals that were positive for spondylitis TB using GeneXpert MTB/RIF rapid method, and 22 samples (31.42%) using culture method (Table 1). Comparison of results between each of the methods is 3:1. The detection of resistances to rifampicin among samples described 4 (6.45%) of 62 positive isolates from GeneXpert method shown resistances to rifampicin, while 5 (22.7%) of 22 positive isolates from culture method shown similar resistances. Based on these results, we observed that samples from individuals suspected with spondylitis TB are still susceptible to rifampicin.

We also compared the validity of GeneXpert MTB/RIF with gold standard examination (MGIT culture) and observed 100% sensitivity and 16.6% specificity rate. Positive predictive value (PPV) and negative predictive value (NPV) are 35.48% and 100%, respectively.

4. Discussion

Molecular diagnostic technique for tuberculosis has proved its efficacy and accuracy to detect the DNA of *M. tuberculosis*.¹⁴ Polymerase chain reaction (PCR) is the most common molecular technique used to detect tuberculosis with sensitivity rate of 80–98%.¹⁹ Various studies observed the results of PCR technique to diagnose extrapulmonary TB.^{8,20} Currently, various PCR studies have developed into GeneXpert MTB/RIF method using heminested real-time polymerase chain reaction (PCR) assay.^{11,17,21} This system works automatically toward the lysis of bacteria, extraction of the DNA, amplifications, and the detection of amplicon in a single system using a rapid approach.¹¹

Sensitivity and specificity of GeneXpert MTB/RIF have been compared with the gold standard method on MGIT medium in which it shown values of 100% and 16.6%, respectively. One of the

Table 1
Comparison between rapid GeneXpert MTB/RIF and MGIT culture for the detection of *M. tb* in vertebral bone tissues.

GeneXpert MTB/RIF	MGIT liquid culture		
	Positive	Negative	Total
Positive	22	40	62
Negative	0	8	8
Total	22	48	70

explanations toward the high sensitivity of GeneXpert method is merely due to no false negative in this study. These results proved that GeneXpert system works consistently with its target. This sensitivity value is similar to several reports.^{11,17,21} However, the specificity value of GeneXpert MTB/RIF is considered low due to the high rate of false positive in this study, especially in several cases where the outcome of *M.tb* culture shown contradictive results with GeneXpert MTB/RIF. The different value between positive and negative samples of spondylitis TB in GeneXpert MTB/RIF and culture is possibly caused by the difference between the sensitivity value from each of the methods. Recent studies explained that GeneXpert has high sensitivity and specificity value ranging from 97–100% for pulmonary specimens.^{11,14} Samples that failed to grow in this medium is caused by the loss of bacteria due to the decontamination procedure and due to the operational procedure of anti-tuberculosis drugs treatment before the surgical approach for spondylitis TB patient that decrease the viability of *M.tb*. This statement is strongly based on personal communication with orthopedics surgeon that described a necessary pre-surgical procedure to give anti-tuberculosis drugs for two weeks as an umbrella therapy for patients suspected with spondylitis TB. The absence of growth in MGIT medium interprets the number of bacteria in samples are $<1 \times 10^7$ CFU/mL. This value represents the minimum numbers of *M.tb*.²³

GeneXpert MTB/RIF can be used as a tool to confirm MDR-TB on suspects. In this study, samples from suspects are still susceptible for rifampicin either on GeneXpert MTB/RIF or DST results on MGIT media. Indeed, we can describe that most of the suspects are not an MDR-TB. A similar result is reported by Ionidis et al.²¹ from pulmonary and extrapulmonary specimens that shown resistance to rifampicin due to a mutation in 95% of *M.tb* in outer hot spot region of the *rpoB* gene.^{21,22}

In this study, we did not conduct the validity test of rifampicin resistance between GeneXpert MTB/RIF and MGIT culture since many of the samples that shown to be resistant in GeneXpert MTB/RIF have negative results in the culture examination. As a reference, Boehme et al.¹⁴ and Hilleman et al.¹¹ reports a high sensitivity and specificity on pulmonary and extrapulmonary samples.

5. Conclusion

Based on the validity test toward the growth of *M.tb*, methods of examination using GeneXpert MTB/RIF can be used as a screening tool for spondylitis tuberculosis due to its high sensitivity and its ability to detect resistance toward rifampicin.

Conflicts of interest

The authors have none to declare.

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