ORIGINAL ARTICLE

Overview of rapid molecular test (RMT)–gene xpert mtb/rif ultra results in pulmonary tuberculosis patients based on the history of therapy

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ABSTRACT

Tuberculosis (TB) is a health threat that remains a problem both globally and nationally. Indonesia has the world's second highest number of TB cases. Various examination methods were developed for detecting this disease; one of the most developed is the Rapid Molecular Test (RMT). This study aims to describe the results of a rapid molecular test (GeneXpert) using the Xpert MTB/RIF Ultra tool in pulmonary tuberculosis based on a treatment history consisting of new cases, relapse cases, and drug withdrawal cases. The study subjects were 47 patients with pulmonary tuberculosis at *Puskesmas Ciwidey*, Bandung Regency Health Office, from February to September 2023. The results showed that characteristics of respondents were male (57.4%), female (42.6%), and mostly were aged between 20 and 44 years (53.1%). These results are in line with previous research. The majority of cases (87.2%) were new cases and the most RMT examination results indicated that MTB detected rifampicin sensitivity (78.7%). In new cases, MTB-detected rifampicin sensitivity at 78.1%, MTB-not detected at 19.5%, and MTB-detected rifampicin resistance at 2.4%. In relapse cases, all respondents (100%) showed MTB detected to be rifampicin-sensitive. In drug withdrawal cases, MTB detected 75% rifampicin sensitivity and 25% rifampicin resistance. The results of this study indicate that one possible factor in the occurrence of germ resistance to antituberculosis drugs is inadequate treatment of TB patients, in this case drug withdrawal cases.

Keyword: Gene xpert MTB/RIF ultra, rapid molecular test, resistance drug, rifampicin, treatment history, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* that can infect the lungs (pulmonary TB) and other parts of the body (extrapulmonary TB), such as the glands, bones and nervous system.[1] This disease continues to pose a significant public health challenge, both nationally and globally. It is estimated that globally, 10.6 million people would suffer from TB in 2021, equivalent to 134 cases per 100,000 people. Among all TB cases, 6.7% are people living with HIV. According to WHO, geographically, the majority of TB cases in 2021 would occur in the Southeast Asia (45%), Africa (23%), and the Western Pacific (18%), with smaller numbers found in the Eastern Mediterranean (8.1%), the Americas (2.9%) and Europe (2.2%).[2] According to the 2016 Global TB Report, Indonesia has the largest TB burden and ranks second with the most TB cases in the world among five other countries: India, China, Nigeria, and Pakistan.[3] Tuberculosis is the leading cause of death from infectious diseases and is one of the ten leading causes of death worldwide.[4]

This disease can be transmitted from one person to another through an airborne droplet from coughing or sneezing. Currently, there is a molecular test to precisely diagnose TB, using a rapid molecular test (RMT) with the polymerase chain reaction (PCR) method, known as GeneXpert. Since 2010, WHO has recommended using the Xpert MTB/RIF tool as an initial examination for diagnosis of TB, drug resistant-TB (DR-TB), and TB in HIV patients.[3,4] The Xpert MTB/RIF examination is a molecular examination with Nucleic Acid Amplification Technology (NAAT) that can diagnose TB with resistance to Rifampicin within 2 hours. The use of rapid molecular tests for TB is regulated by Minister of Health Regulation No. 67 of 2016 concerning TB management and the National Action Plan for TB Management through Strengthening the TB Laboratories 2016-2020.[3,5]

This study aims to describe the results of a rapid molecular test GeneXpert using the Xpert MTB/RIF Ultra tool in pulmonary tuberculosis based on its treatment history. This includes new cases, relapse cases, and drug withdrawal cases.

METHODS AND SUBJECT
Research Design

The research design used was observational cross sectional to determine the results of rapid molecular test examination or GeneXpert using the Xpert MTB/RIF Ultra tool in pulmonary tuberculosis based on its treatment history consisting of new cases, relapse cases, and drug withdrawal cases.

Research Subjects, Sampling and Method

The study population consisted of all patients diagnosed with pulmonary tuberculosis by doctors at the *Puskesmas Ciwidey*, Bandung Regency, from February to September 2023. The criteria for diagnosing TB patients in this study were based on strongly supported clinical symptoms. The study was conducted on patients diagnosed with pulmonary tuberculosis at the *Puskesmas Ciwidey* in Bandung Regency, from February to September 2023, depending on certain inclusion and exclusion criteria. This study included patients who had been clinically diagnosed with pulmonary tuberculosis and participated as respondents. In this study, pulmonary tuberculosis patients transferred from other health facilities were excluded, as were pulmonary tuberculosis patients who underwent the RMT examinations at other health facilities. The subjects of this study were selected using the total sampling method, resulting in a sample size of 47 patients who fulfilled the research criteria.

Research Procedure

Subjects were selected according to the inclusion and exclusion criteria. The study's implementation began with the researcher explaining to the patients the purpose and objectives of the examination. Patients who were willing to participate in the study were required to fill out an informed consent sheet, which had to be signed.

Tuberculosis Testing Using Rapid Molecular Tests
Method and RMT Testing Materials

In this study, RMT testing with Xpert MTB/RIF is a molecular detection method based on nested real-time PCR. The PCR primers used are capable of amplifying about 81 base pairs of the core region of the MTB complex's rpoB gene, while the probes are designed to distinguish wild-type sequences and mutations in the core region associated with resistance to rifampicin.[3,6]

This is done with the GeneXpert tool, which uses an automated system that integrates the processes of specimen purification, nucleic acid amplification, and target sequence detection. The system consists of a GeneXpert device, a computer, and software. Each test uses disposable cartridges and is designed to minimize cross-contamination. Xpert MTB/RIF cartridges also have Sample Processing Control (SPC) and Probe Check Control (PCC). The sample processing control effectively control the target bacteria and monitor the presence of PCR reaction inhibitors, while PCC ensures the reagents rehydration process, catridge PCR tube filling, probe integrity, and dye stability. Examination materials in RMT can be sputum or non-sputum, such as cerebrospinal fluid (CSF), gastric lavage, other body fluids, lymph nodes, tissues, and feces. In this study, sputum was used as the test material.[3,6,7]

Principle of RMT Testing

The Xpert MTB/RIF test can detect complex MTB and rifampicin resistance simultaneously by amplifying the specific sequence of the rpoB gene of the complex MTB using five molecular beacons (probes A - E) to detect mutations in the rpoB gene region. Each molecular beacon was labelled with a different fluorophore dye. The maximum cycle threshold (Ct) valid for the analysis of results on probes A, B, and C is 39 cycles, while on probes D and E it is 36 cycles.[3] In this study, we used that principle.

RMT Examination Results

The results of the RMT examination can be interpreted as follows:
1. 'MTB detected' if two probes provide Ct values within valid limits and delta Ct min (the smallest Ct difference between probe pairs) < 2.0.
2. 'Rifampicin Resistant not detected' if delta Ct max (difference between the earliest appearing probe and the latest appearing probe) ≤ 4.0
3. 'Rifampicin Resistant detected' if delta Ct max > 4.0
4. 'No MTB detected' if there is only one or no positive probe.

The Xpert MTB/RIF test is automatically set according to the Xpert MTB/RIF working protocol and cannot be modified by the user.[3]

Data Analysis

The data obtained is presented in tabulated form:

Univariate is a frequency table that describes the characteristics of each research variable: age, gender, type of pulmonary tuberculosis based on treatment history, and the frequency distribution of RMT GeneXpert examination results. It describes the frequency distribution of RMT GeneXpert examination results in pulmonary tuberculosis patients based on treatment history (new cases, relapse cases, drug withdrawal cases).

Ethical Aspects of Research

The researcher has obtained a research permit from the Head of *Puskesmas Ciwidey* with letter number B/0238/423.8/I/2023/PKMCWD. The name of the research subject is not included in the data sheet, but only given as a code. The researcher guarantees that all information obtained from the research subject is kept confidential.

RESULT AND DISCUSSION
Results
Respondent Characteristics

In this study, respondent characteristics included gender, age, type of pulmonary tuberculosis case based on treatment history, and RMT examination results displayed in percentage form. Table 1 describes the results of respondent characteristics in pulmonary tuberculosis patients.

Table 1. Description of Respondent Characteristics

Variables	Number of Respondents	Percentage (%)	Total (%)
Gender			
Male	27	57,4	
Female	20	42,6	47 (100%)
Age			
10 - 19 years old	5	10,6	
20 - 44 years old	25	53,1	
45-59 years old	10	21.3	47 (100%)
$\geq$ 60 years old	7	15	
Types of Pulmonary Tuberculosis Cases			
New Case	41	87,2	
Relapse Case	2	4,3	47 (100%)
Drug withdrawal Case	4	8,5	
RMT Examination Result			
MTB Detected Rifampicin Sensitive	37	78,7	
MTB Detected Rifampicin Resistance	2	4,3	47 (100%)
MTB Not Detected	8	17	

Frequency Distribution of RMT Examination Results (Genexpert)

The frequency distribution of RMT (Genexpert) examination results in pulmonary tuberculosis patients based on their treatment history (new cases, relapse cases, drug discontinuation cases) can be seen in Table 2.1.

Table 2. Frequency distribution of RMT (GeneXpert) examination results in pulmonary tuberculosis patients based on their treatment history (New Case, Relapse Case, Drug Withdrawal Case).

	New Case	Recurrence Case	Drug withdrawal case
MTB Not Detected	19,5 %	0	0
MTB Detected Rifampicin Sensitive	78,1 %	100 %	75 %
MTB Detected Rifampicin Resistance	2,4 %	0	25 %
Total	100 %	100 %	100 %

Discussion

Table 1 indicates a higher number of male respondents compared to female respondents. According to the Basic Health Research data of the Ministry of Health of the Republic of Indonesia in 2018 *(RISKESDAS 2018),* the prevalence of pulmonary tuberculosis based on gender characteristics shows a similar thing: men more than women in a ratio of 5:4.[8] Hartiyah L et al. conducted a similar study in 2021, which described the results of the Mycobacterium Tuberculosis molecular rapid test examination at Ulin Banjarmasin Hospital in 2020-2021. In this study, it was stated that the incidence of pulmonary tuberculosis was more prevalent in men (61.5%) than in women (38.5%), and men had a risk of developing pulmonary tuberculosis 1.6 times higher than women.[9] There are several groups of people who have a higher risk of developing TB disease: people who are HIV positive and other immunocompromised diseases; people who take immunosuppressant drugs for a long time; smokers; people with high alcohol consumption; children <5 years old and elderly; people have close contact with people with active infectious TB disease; people who are in places with a high risk of tuberculosis infection (for example: correctional institutions, long-term care facilities), and health workers.[1] Men are more likely to suffer from pulmonary tuberculosis, one of which may be due to behavior differences: smoking habits in men are 41.6 percent higher than in women.[10]

The age division in this study is based on the age division of the Ministry of Health of the Republic of Indonesia.[11] The results revealed that the the majority of respondents were adult, aged 20-44 years (53.1%). This is in line with the data from the Global TB Report 2022, which showed that the productive age group has the highest number of TB cases, especially those aged 25 to 34 years. In Indonesia, the highest number of TB cases is in the productive age group, especially at the age of 45 to 54 years. At this age, most people work and interact with others, increasing transmission risk.[12]

Based on treatment history, pulmonary tuberculosis is classified into new cases, cases with treatment history, relapse cases, treatment cases after failure, cases after loss of follow-up or drug withdrawal, other cases, and cases with an unknown treatment history. In this study, pulmonary tuberculosis cases included new cases, relapse cases, and drug withdrawal cases. The new cases were patients who had never received anti-tuberculosis drugs before or a history of getting anti-tuberculosis drugs for less than 1 month (< 28 doses if using program drugs). Relapse cases are patients who have previously received anti-tuberculosis drugs and were declared cured or completed treatment at the end of treatment and currently have a diagnosis of TB re-episode (due to reactivation or a new episode caused by reinfection). Patients who had taken antituberculosis drug for 1 month or more and did not continue for more than 2 consecutive months were called dropout cases.[1,5,13] The results showed that the new cases were the most common type of pulmonary tuberculosis cases in this study. The National Strategy for TB Elimination is contained in Presidential Regulation Number 67 of 2021 concerning Tuberculosis Control. The government has committed to various strategies to overcome TB in Indonesia, which include tracking TB cases, strengthening commitment, increasing access to TB services, optimizing TB promotion and prevention efforts, TB treatment and infection control through the utilization of research and technology.[13] Based on this, it is possible that the number of new TB cases may continue to persist, despite a decrease in drug withdrawal and relapse cases.

In cases of pulmonary tuberculosis, the RMT examination results can be MTB Detected Rifampicin Sensitive, MTB Detected Rifampicin Resistance, and MTB Not Detected.

The most common results in this study were MTBetected Rifampicin Sensitive at 78.7%. One of the supporting examinations for the diagnosis of TB is the RMT examination with Xpert MTB/RIF.[3,6] This examination is a molecular detection method based on nested real-time PCR. The Xpert MTB/RIF examination is able to qualitatively detect complex MTB DNA from direct specimens, both sputum and non-sputum. In addition to detecting MTB complex, the Xpert MTB/RIF test, using Xpert MTB/RIF Ultra, also detects mutations in the rpoB RNA gene (ribonucleic acid polymerase β subunit) and silent mutations that cause resistance to rifampicin. The Xpert MTB/RIF test can diagnose TB and rifampicin resistance quickly and accurately.[3,6] The sensitivity and specificity of the Xpert test for detecting rifampicin resistance are 98.2% and 97.0%, respectively.[7,14] The GeneXpert system provides results through fluorescence signal measurement and an automated calculation algorithm.[3,18] The RMT results will indicate the presence or absence of Mycobacterium tuberculosis complex DNA as well as the presence or absence of mutations encoding rifampicin resistance. The RMT results provide accurate results and may allow the initiation of treatment for multidrug-resistant tuberculosis.[19] MTB Detected Rifampicin Resistance reveals the existence of both and the rpoB gene mutation, indicating a significant likelihood of rifampicin resistance. If such a result is found, the management continues according to the flow of diagnosis of drug-resistant tuberculosis (TB). MTB Detected rifampicn-sensitive result means the MTB DNA is detected, yet the rpoB gene mutation and silent mutation are not detected. The patient is most likely to have a sensitivity to rifampicin; therefore, the patient's management should be continued according to the usual TB diagnosis flow. MTB not detected means that MTB DNA

is not detected.[3] Some RMT examination yielded invalid results, due to incorrect processing of the sample or interference during the RMT examination process. On the other hand, error results arise from system due to component failure during the RMT examination process, which prevents the detection of MTB and the determination of the examination results. In these circumstances, the GeneXpert test must be repeated.[3]

Drug-resistant tuberculosis (DR-TB) remains a threat to TB control and is one of the major public health problems in many countries around the world. Globally in 2019, an estimated 3.3% of new TB patients and 17.7% of treated TB patients were drug-resistant TB patients.[20] These data show results that align so closely to the findings of this study. The main factor causing germ resistance to antituberculosis drugs is inadequate or non-standard treatment management for TB patients.[20] This is the evident in this study: 25% of drug withdrawal pulmonary tuberculosis cases had resistance to rifampicin. In 2019, there were an estimated 9.96 million TB incidences worldwide, of which 465,000 were multidrug-resistance TB (MDR TB) or rifampicin-resistance TB (RR TB). Of the estimated 465,000 drug-resistant TB patients, only 206,030 were found and 177,099 (86%) treated, for a global treatment success rate of 57%.[20] The diagnosis of pulmonary TB in adults must first be established by bacteriological examination. Bacteriological examinations include microscopic examination, RMT, and culture. RMT examination is used to confirm TB diagnosis, while microscopic examination is still used to monitortreatment progress.[3] In addition to confirming the diagnosis, RMT results are also useful for determining the most suitable therapy for the patients. All patients who have not been previously treated and have no risk factors for drug resistance should receive WHO-approved first-line treatment using quality-assured drugs. The intensive phase should

include two months of treatment using isoniazid, rifampicin, pyrazinamide, and ethambutol. In the continuation phase, isoniazid and rifampicin should be given for 4 months.[5] The treatment strategy for patients with DR-TB is to ensure that all patients with confirmed RR/MDR TB have access to rapid, standardized and quality treatment. The drug mix for patients with DR-TB consists of first-line and second-line antituberculosis drug. The antituberculosis drug mix can be adjusted if there is a change in MTB sensitivity test results. The decision to change is made by the DR-TB clinical expert team. In accordance with WHO recommendations in 2020, DR-TB treatment in Indonesia currently uses an injectable drug-free combination, which is divided into two, short-term (9-11 months) and long-term (18-20 months) treatment combinations.[20] The classification of DR-TB drugs is based on in-depth studies conducted by the WHO regarding their benefits and side effects. These drugs include Levofloxacin/Moxifloxacin, Bedaquiline, Linezolid, Clofazimine, Cycloserine or Terizidone.[20]

The results of this study indicate that inadequate treatment of TB patients, specially drug withdrawal cases, may contribute to the occurrence of germ resistance of antituberculosis drugs, resulting in a high rate of MTB Detected Rifampicin Resistance (25%). In new cases, where patients have never received antituberculosis drugs before, and in relapse cases with an adequate treatment history, almost all respondents reported positive results for RMT MTB Detected Rifampicin Sensitive. In other words, first-line treatment in TB cases in the form of Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol can still be given. This study indicate that one possible factor in the occurrence of germ resistance to antituberculosis drugs is inadequate treatment of TB patients, in this case drug withdrawal cases.

The research's limitation is that the number of respondents in each case group is not evenly distributed. Apart from that, determining the diagnosis of tuberculosis is only based on clinical symptoms. Further research suggests that diagnosing tuberculosis patients should not rely solely on clinical symptoms, but also include supporting examinations such as radiology or microbiology.

CONCLUSION

Based on the study's results, the majority of respondents were male (57.4%), female (42.6%), and aged between 20 and 44 years (53.1%) The majority (87.2%) were new cases, and the most RMT examination results MTB detected rifampicin sensitive 78.7%. Additionally, in new cases, MTB was found to be sensitive to rifampicin in 78.1% cases, not detectable in 19.5% cases, and resistant to rifampicin in 2.4% cases. In relapse cases, all respondents (100%) showed MTB-detected rifampicin-sensitive. In drug withdrawal cases, MTB detected 75% rifampicin-sensitive, and 25% rifampicin-resistance. The results of this study indicate that one possible factor in the occurrence of germ resistance to antituberculosis drugs is inadequate treatment of TB patients, in this case drug withdrawal cases.

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DECLARATION OF INTERESTS

The authors declare that there is no conflict of interest in all parties involved both with patients and other parties in this study.

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