Abstract

Background: Tuberculosis (TB) is the fourth leading cause of death in Indonesia. In 2017, the World Health Organization (WHO) estimated that only 2% of the TB patients in Indonesia had only been tested with rapid diagnostics at the time of diagnosis, resulting in largely undetected rifampicin-resistant TB (RR-TB). Utilization of GeneXpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) assay as a point-of-care molecular assay to detect TB and RR-TB and serving its purpose in accordance with the first pillar of the WHO End-TB Strategy. Objective: This study investigated the use of GeneXpert MTB/RIF assay to determine the molecular epidemiology of RR-TB in an urban setting of Indonesia. Methods: All molecular epidemiological and microbiological databases were retrospectively examined from GeneXpert MTB/RIF assay results in Siloam Hospital Lippo Village. The sociodemographic characteristics and results of microbiological examinations consisting of Ziehl–Neelsen staining and GeneXpert MTB/RIF assay were analyzed. Results: During the study period, 600 cases were registered, and GeneXpert MTB/RIF tests were done in which the tests yielded 597 (99.5%) valid results; 62.0% were male and adult of age category; of whom 29 samples (4.9%) were found to be RR-TB, 186 samples (31.2%) were RIF sensitive, and remainders were negative. Conclusions: The results of GeneXpert MTB/RIF to be a fundamental diagnosis of RR-TB and subsequently to notify MDR-TB were satisfying and valuable in this study. This further increased and reinforced TB surveillance and national TB program to finally bring about WHO end-TB strategy one step closer in Indonesia.

Keywords: End-TB Strategy, GeneXpert, MTB/RIF, Indonesia, resistance, rifampicin

Introduction

Tuberculosis (TB) is a communicable disease caused by intracellular bacteria from the genus Mycobacterium with the species of Mycobacterium tuberculosis (MTB) that has caused one of the health burdens in the world, being the tenth leading cause of death worldwide.[1,2] Globally, in 2017, according to the World Health Organization (WHO), it is estimated that there were 1.3 million deaths from TB among HIV-negative patients with additional 300,000 deaths from TB among HIV-positive people. Nationally, Indonesia ranked number 3 in the 30 high TB burden countries, and is estimated to have mortality of 107,000 among HIV-negative people and 9,400 among HIV-positive people.[3] Most of these deaths could be prevented with early recognition and appropriate treatment.
for short-course regimens with high cure rates, with RIF being arguably the most important drug in the treatment of TB. In 2017, globally, there were an estimated 558,000 new cases of rifampicin-resistant resistant TB (RR-TB), with an estimated 82% having multidrug-resistant-TB (MDR-TB) and nationally, Indonesia contributed 12,000 cases of estimated MDR/RR-TB cases among notified pulmonary TB (PTB) cases, with 2.4% of TB cases with MDR/RR-TB among new cases and 13% among previously treated cases. Detection of drug(s) resistant TB has been traditionally burdensome requiring lengthy time for cultures, drug susceptibility tests (DSTs), sophisticated biosafety, and laboratory infrastructures. Nevertheless, drug resistant, in particular RR-TB, can be detected rapidly with GeneXpert MTB/RIF assay, which is the newest method endorsed by the WHO able to detect RR-TB in a short period of time with high sensitivity and specificity. In Indonesia, only a few published reports have been published, and data concerning molecular epidemiology of RR/MDR-TB in the province scale were limited nor made readily accessible. This study aimed to use the data from GeneXpert MTB/RIF assay to determine the molecular epidemiology of RR-TB in an urban setting of Banten province, Indonesia.

**Methods**

According to the Guidelines for National TB Control (Pedoman Nasional Pengendalian Tuberkulosis) algorithm for diagnosing TB, TB is to be diagnosed essentially with clinical examinations by physicians and sputum or GeneXpert MTB/RIF (Cepheid®) assay test. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Pelita Harapan (Ref: 151/K-LKJ/ETIK/II/2019). Data were anonymized before analysis by de-identifying patient data.

**Study design and population**

The study was performed in Banten Province, Indonesia. This was a retrospective study enrolling 600 samples which were based on the database of epidemiological data and microbiological data of patients who were tested for RR from the hospital database of all samples processed with the GeneXpert MTB/RIF assay from November 2017, when the utilization of GeneXpert MTB/RIF began, to June 2019. The population of the study included patients who were referred to the Siloam Hospital's microbiological laboratory from out-/inpatient in hospitals in Banten province.

**Variable definitions**

Variable definitions of TB were made adapting from the WHO definitions in terms of anatomical site of TB disease and history of previous treatment: patient registration group.

**Tuberculosis case**

Diagnosis of TB case was determined by specialists, based on the criteria with a minimum of one out of the three criteria: (1) Signs and symptoms of TB established by physicians; (2) MTB complex identified positively from a clinical specimen for smear examinations of acid–fast bacilli (AFB); and/or (3) by GeneXpert MTB/RIF assay positivity.

**Pulmonary tuberculosis**

A patient with a TB case involving the lung parenchyma. Miliary TB is included as PTB. A patient with PTB and extra PTB (EPTB) should be classified as a case of PTB.

**Extrapulmonary tuberculosis**

A patient with a TB case involving organs other than the lungs, for example, lymph nodes, abdomen, skin, joints and bones, meninges, and pleura. Diagnosis should be based on strong clinical evidence consistent with active EPTB or at least one specimen with confirmed MTB or histological.

**New patients**

A patient with a TB case who never received treatment for TB, or have taken anti-TB drugs for <1 month, may have positive or negative bacteriological specimens, and may have disease at any anatomical site.

**Previously treated patients**

A patient with a TB case who have received 1 month or more of anti-TB drugs in the past, may have positive or negative bacteriological specimens, and may have disease at any anatomical site.

**Other**

A patient with a TB case that does not fit the above definitions, such as patients who do not know whether they have been previously treated, previously treated but with unknown outcome of that previous treatment, and/or who have returned to treatment with smear-negative PTB or bacteriologically negative EPTB.

**Bacteriologic examinations and identifications**

After registration, the samples were sent to the microbiological laboratory at the Central Laboratory of Siloam Hospitals Lippo Village, which is one of the reference laboratory centers for GeneXpert MTB/RIF assay in Banten Province. All the specimens were done for Ziehl–Neelsen (ZN) staining using the ZN method and GeneXpert MTB/RIF assay following the instruction guide from the company. Sputum AFB smear test results were reported semi-quantitatively and were interpreted using the International Union against Tuberculosis and Lung Disease. Results of GeneXpert MTB/RIF assays were reported and collected qualitatively.

**Data collection and analysis**

The data collected for the study consisted of (1) sociodemographic characteristics derived from patient's family certificate, consisting of their sex, age, address, education level and job, (2) type of samples for examination, (3) national TB registry form, and/or (4) GeneXpert MTB/RIF test request forms, diagnosis in terms of site of disease, type of patient (new-, previously treated patients, or others). Results of the ZN staining and GeneXpert MTB/RIF test were entered into Excel files. Statistical analysis was performed using SPSS Version 21.0 (IBM Corp., Released 2012, Armonk, NY).
RESULTS

Characteristics of the study population

During the study period, from the total of 600 samples that were collected and processed, the assay provided valid results in 597 (99.5%) samples and unsuccessful results in 3 (0.5%) samples. Hence, the total number of samples eligible in the research are 597 samples. The sociodemographic aspect of the samples gave results of a mean age (range) of 47.91 (1–90) years and a median of 49 years. Majority of the samples were adult (61.1%) and male (62.0%). Regarding their educational status, most samples had a secondary education (52.6%), with occupational status of the samples predominated by white-collar jobs (34.0%). Anatomically, most samples came from PTB (77.6%) samples, whilst new cases (56.3%) of TB dominated the treatment history group [Table 1].

Outcomes of GeneXpert Mycobacterium tuberculosis/ rifampicin assay tests and microscopy

The GeneXpert MTB/RIF assay test for the detection of MTB was positive in 382 (64.0%) samples and negative in 215 (36.0%) samples; 82.3% of the positive samples were with positive ZN staining and 3.6% of the positive GeneXpert MTB/RIF assay tests were negative on ZN staining [Table 2].

Epidemiology rifampicin-resistant Mycobacterium tuberculosis and characteristics of samples

During the study period, the number of samples with RR-TB varied from year to year. Of the 36.0% of cases with a positive GeneXpert MTB/RIF assay test, 4.9% showed RR. The rate of resistance was 0.0% in 2017, 8.6% in 2018, and 1.0% in 2019 [Figure 1]. Resistance was observed primarily in male (65.5%) and adult age group (69.0%) within Banten area (86.2%) followed by outside and unspecified area (6.9%, respectively). Education status of resistance samples was mostly secondary education (48.3%) with employment status as white-collar jobs (34.5%) as well as unemployed (31.0%) being the top contributors. Majority of the diagnoses were contributed from PTB samples (82.8%) and found most frequently in new cases (48.3%) followed by previously treated (34.5%) and other (17.2%).

DISCUSSION

This study has identified data of sociodemographic and epidemiology of MTB and RR-TB identification by ZN staining and GeneXpert MTB/RIF assay test. According to the Guidelines for National TB Control (Pedoman Nasional Pengendalian Tuberkulosis) algorithm for diagnosing TB, GeneXpert MTB/RIF assay test will be chosen in terms of accessibility to facilities in health-care facilities or in patients

| Table 1: Baseline characteristics of 597 samples (n=597) |
|---------------------------------------------------------|
| Characteristic(s)                                      | n (%)  |
| Sex                                                    |        |
| Female                                                 | 227 (38.0) |
| Male                                                   | 370 (62.0) |
| Age group classification                               |        |
| Infant and child                                       | 11 (1.8)  |
| Young adult                                            | 51 (8.5)   |
| Adult                                                  | 365 (61.1)  |
| Geriatric                                              | 153 (25.6)  |
| Unspecified                                            | 17 (2.8)    |
| Locality                                               |        |
| Banten                                                 | 462 (77.4)  |
| Outside                                                | 112 (18.8)  |
| Unspecified                                            | 23 (3.9)    |
| Educational background                                 |        |
| Noneducation                                           | 25 (4.2)    |
| Primary                                                | 137 (22.9)  |
| Secondary                                              | 314 (52.6)  |
| Tertiary                                               | 83 (13.9)   |
| Unspecified                                            | 38 (6.4)    |
| Job                                                    |        |
| Unemployed                                             | 171 (28.6)  |
| Blue-collar worker                                     | 36 (6.0)    |
| White-collar worker                                    | 203 (34.0)  |
| Entrepreneur                                            | 101 (16.9)  |
| Students                                               | 43 (7.2)    |
| Others                                                  | 43 (7.2)    |
| Anatomical site of TB                                  |        |
| PTB                                                    | 463 (77.6)  |
| EPTB                                                   | 25 (4.2)    |
| Unspecified                                            | 109 (18.3)  |
| Type of case                                            |        |
| New case                                               | 336 (56.3)  |
| Previously treated                                     | 146 (24.3)  |
| Other                                                  | 115 (19.3)  |

Figure 1: Tabulation and Graph of GeneXpert mycobacterium tuberculosis/rifampicin assay tests, 2017–2019

| Table 2: Results of GeneXpert test and Ziehl-Neelsen staining for the detection of Mycobacterium tuberculosis |
|-------------------------------------------------------------------------------------------------------|
| ZN staining | GeneXpert MTB/RIF assay test |
| Positive, n (%) | Negative, n (%) | Total, n |
| Positive | 377 (82.3) | 81 (17.7) | 458 |
| Negative | 5 (3.6) | 134 (96.4) | 139 |
| Total | 382 (64.0) | 215 (36.0) | 597 |

MTB: Mycobacterium tuberculosis, ZN: Ziehl-Neelsen, RIF: Rifampicin
with a history of TB treatment, history of close contact with drug-resistant TB, and/or HIV patients. Nonetheless, the GeneXpert MTB/RIF assay test has been able to provide the diagnosis of TB within a short period of time and was able to provide results that were sufficiently reliable and accurate in terms of sample needed. The GeneXpert MTB/RIF assay test required a minimum of 100 cells/mL sputum compared to ZN staining which required a minimum of 10,000 cells/mL sputum to recognize MTB or to be positive. GeneXpert MTB/RIF assay is also recommended by the WHO for its use as a point-of-care molecular assay, indicating that these results can be considered sufficient in the absence of complementary conventional or molecular tests.

Males dominated the study with 62.0%, as seen in other studies, in which sex was found to be statistically not significant as shown also in our study [Table 3]. Sociodemographic characteristics of age have shown the cases of TB referred for verification were in patients of all ages, likewise elsewhere in regions of Indonesia, ranging from 0 to 65 + years, with the most referred patients belong in the adult age category. This is in line with the trend of rapid aging of the Indonesian population, and similar ranges of TB cases have been reported from various researches and similar results have shown in our study.

In our study, the urban area was found to be statistically insignificant as evidenced in other studies. The result is contradictory toward other studies in which urban area carries significance in risk of developing RR/MDR-TB for it could be interpreted as urban areas of having better houses – where walls, roofs, and windows are built with more solid materials, are well less ventilated, and therefore increase the risk of within-household transmission. Another possible pathway is increased risk of household crowding because those with better houses may spend more time indoors to socialize.

Table 3: Sosiodemographic Characteristic of GeneXpert MTB/RIF Assay Test

| MTB Not Detected, No. (%) (n=382) | Rifampicin sensitive, no. (%) (n=186) | Rifampicin resistant, no. (%) (n=29) | 95% confidence interval | P |
|----------------------------------|----------------------------------------|--------------------------------------|-------------------------|---|
| Sex                              |                                       |                                      |                         |   |
| Female                           | 156 (68.70%)                          | 61 (26.90%)                          | 10 (4.40%)              | 0.345-1.684 0.166 |
| Male                             | 226 (61.10%)                          | 125 (33.80%)                         | 19 (5.10%)              |   |
| Age                              |                                       |                                      |                         |   |
| Infants & Children               | 9 (81.80%)                            | 2 (18.20%)                           | 0 (0.00%)               | 0.009 |
| Teenagers                        | 27 (52.90%)                           | 21 (41.20%)                          | 3 (5.90%)               | 0.081-3.795 |
| Adult                            | 222 (60.80%)                          | 123 (33.70%)                         | 20 (5.50%)              | 0.534-7.079 |
| Geriatric                        | 114 (74.50%)                          | 36 (23.50%)                          | 3 (2.00%)               | 0.426-4.509 |
| Unspecified                      | 10 (58.80%)                           | 4 (23.50%)                           | 3 (17.60%)              | 0.233-2.670 |
| Locality                         |                                       |                                      |                         |   |
| Banten                           | 295 (63.90%)                          | 142 (30.70%)                         | 25 (5.40%)              | 0.463 |
| Outside                          | 74 (66.10%)                           | 36 (32.10%)                          | 2 (1.80%)               | 0.317-1.930 |
| Not Specified                    | 13 (56.50%)                           | 8 (34.80%)                           | 2 (8.70%)               | 0.301-2.078 |
| Education Status                 |                                       |                                      |                         |   |
| Non Education                    | 18 (72.00%)                           | 7 (28.00%)                           | 0 (0.00%)               | 0.956 |
| Primary                          | 86 (62.80%)                           | 42 (30.70%)                          | 9 (6.60%)               | 0.255-2.371 |
| Secondary                        | 202 (64.30%)                          | 98 (31.20%)                          | 14 (4.50%)              | 0.445-2.142 |
| Tertiary                         | 52 (62.70%)                           | 27 (32.50%)                          | 4 (4.80%)               | 0.466-2.021 |
| Unspecified                      | 24 (63.20%)                           | 12 (31.60%)                          | 2 (5.30%)               | 0.451-2.392 |
| Job                              |                                       |                                      |                         |   |
| Unemployed                       | 129 (75.40%)                          | 33 (19.30%)                          | 9 (5.30%)               | 0.050 |
| Blue-Collar Worker               | 23 (63.90%)                           | 11 (30.60%)                          | 2 (5.60%)               | 0.192-0.833 |
| White-Collar Worker              | 120 (59.10%)                          | 73 (36.00%)                          | 10 (4.90%)              | 0.288-1.940 |
| Entrepreneur                     | 61 (60.40%)                           | 37 (36.60%)                          | 3 (3.00%)               | 0.476-1.898 |
| Students                         | 24 (55.80%)                           | 16 (37.20%)                          | 3 (7.00%)               | 0.448-2.004 |
| Others                           | 25 (58.10%)                           | 16 (37.20%)                          | 2 (4.70%)               | 0.427-2.539 |
| Anatomical Site of TB            |                                       |                                      |                         |   |
| PTB                              | 287 (62.00%)                          | 152 (32.80%)                         | 24 (5.20%)              | 0.329 |
| EPTB                             | 19 (76.00%)                           | 6 (24.00%)                           | 0 (0.00%)               | 0.893-2.313 |
| Unspecified                      | 76 (69.70%)                           | 28 (25.70%)                          | 5 (4.60%)               | 0.311-2.365 |
| Types of Cases                   |                                       |                                      |                         |   |
| New Case                         | 215 (64.00%)                          | 107 (31.80%)                         | 14 (4.20%)              | 0.535 |
| Previously Treated               | 88 (60.30%)                           | 48 (32.90%)                          | 10 (6.80%)              | 0.788-2.041 |
| Other                            | 79 (68.70%)                           | 31 (27.00%)                          | 5 (4.30%)               | 0.807-2.396 |

CI: Confidence interval, TB: Tuberculosis, PTB: Pulmonary TB, EPTB: ExtraPTB MTB: Mycobacterium tuberculosis, RIF: Rifampicin
Occupational/socioeconomic and educational status have mixed evidence from other studies on whether higher socioeconomic and educational status is associated with a reduced or increased trend of TB, notably MDR-TB. In our study, socioeconomic status showed statistically significant association, whereas education did not, as confirmed by different literature in different countries. The reason in which the high number of TB cases found in workers, especially white-collar jobs, may lay in the new policy implemented by the Indonesian government through regulation of national health-care insurance which workers are to be enrolled in the health-care insurance effective per 2015, giving better chances in a better healthcare-seeking behaviour among workers. TB percentage in white-collar workers was higher compared to other jobs as supported by studies in India. Our study has several limitations. First, our findings may not be able to fully representative of Indonesia nor Banten province. Second, we did not perform mycobacterial culture nor DSTs. The resistance could be due to repeated and inappropriate ways of taking the medication or treatment dropout that made the bacteria mutate and develop resistance against the drugs. Patients with nonadherence to treatment may remain infectious, experience increased risk of TB recurrence, TB-related mortality or increased probability of resistance. Thus, prompting adherence to treatment and further shorten the time needed for diagnosis to reduce the chain of infection and resistance. Nevertheless, our study showed that patients with previous TB status did not show association with the development of RR-TB. This could be based on (1) primary transmission of MDR-TB strains is ongoing, (2) the low mutation rate of MTB in vitro but higher in the host in which host genetic predisposition of in vivo environment that might drive mutagenesis acting as the basis for the development of MDR-TB with spontaneous chromosomally-borne mutation in MTB at predictable rates are thought to confer resistance to anti-TB drugs as the major underlying event, (3) failure to recognize nontuberculous Mycobacteria causing condition similar of that caused by MTB, and (4) our results may be limited by the definition of MDR-TB that combined patients confirmed with culture and DST which detect about 95-98% of MDR-TB compared to those tested with GeneXpert MTB/RIF alone.

The overall prevalence of RR-TB throughout the study was 4.80%, contributing approximately 31.20% compared to the reported national RR/MDR-TB in Indonesia (15.40%) during the year of 2017. The increase in the overall prevalence of detected cases are contributed due to Indonesia advancement in TB surveillance and control by the implementation and achieving better recording of case through nation-wide coverage of the electronic recording and reporting system in National Tuberculosis Program (NTP) facilities, introduction of mandatory TB notification, establishment of public-public and public-private engagement schemes, improvement of health sector performance and the enforcement of the usage of GeneXpert MTB/RIF assay made available with Regulation of Minister of Health (MoH) issued in 2016. These efforts were based on the high burden of TB in Indonesian society in which annually, TB affects an estimation of 4 million people in the WHO South-East Asia (SEA) Region contributing for 41% of estimated global TB cases with most deaths reported from Bangladesh, India, Indonesia, Myanmar, and Thailand and since 2011, TB has been the primary cause of death from a single infectious agent, topping above HIV/AIDS. This further heightens the alertness for the detection of TB and subsequently drug resistant TB, either RR/MDR-TB or Extensively Drug Resistant TB (XDR-TB), with emphasis of RIF holds its importance as one of the most effective anti-TB drugs due to its effective against actively-and slow-metabolizing bacilli contributing as primary component of the current first-line treatment regimen. In addition, RIF monoresistance is rare as RIF resistance occurs concomitantly with resistance towards other drug, mostly associated with INH, making RIF targets a surrogate marker or proxy for MDR phenotype and the need of accurate drug resistance surveillance data. Further understanding of drug resistant TB can be achieved in line for uses to assess and improve NTP, initiating effective therapy as soon as possible, preventing drug resistant TB through accurate determination of patient requiring treatment and follow-up for TB, and leading to eradication of this disease nationwide and worldwide.
Thus, we could not estimate the proportion of false positive and negative results of the Xpert assay to diagnose TB or RR-TB compared with the gold standard. Third, rpoB gene sequencing was not done, so we could not establish the specific rpoB mutations nor, therefore, the specificity and sensibility of the assay to detect mutations in the rpoB gene. Lastly, the availability of certain data, such as the mode of TB contact and other characteristics or risk factors was limited due to the retrospective manner and relied on collected data.

To our best knowledge, this is the first study to report on the molecular epidemiology of RR–TB in the province scale in Indonesia. Many patients with RR-TB.

**Conclusions**

The risk factor associated for RR/MDR-TB among patients is employment status. Results of GeneXpert MTB/RIF to be a paramount for diagnosis of RR-TB and subsequently to notify MDR-TB were satisfying and valuable in urban setting of Banten province. This further increased and augmented TB surveillance and national TB program to finally bring about WHO End TB Strategy one step closer in Indonesia.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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