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## Original Article

### First Line Anti-Tuberculosis Drug Resistance Pattern in Multidrug-Resistant Pulmonary Tuberculosis Patients Correlate with Acid-Fast Bacilli Microscopy Grading

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#### ABSTRACT

Multidrug-resistant tuberculosis (MDR-TB) is a global public health crisis. Acid-fast bacilli (AFB) gradation in sputum examination is an important component in Pulmonary Tuberculosis (PTB) diagnosis and treatment outcome monitoring. Previously treated pulmonary TB patients with a higher AFB smear gradation may have higher rates of acquired resistance. Patients with a higher AFB grade indicate a higher bacillary load and had higher rates of acquired resistance. This study aims to evaluate the correlation between AFB gradation and first-line anti-TB drug resistance patterns in MDR pulmonary TB patients. This was a retrospective study conducted from August 2009 to April 2018 in Dr. Soetomo Hospital. Sputum samples were taken from MDR PTB patients. Sputum smear examination was done using Ziehl–Neelsen staining and gradation was measured according to IUATLD criteria. Samples with positive smear were evaluated for resistance patterns based on culture and resistance tests using the MGIT 960 BACTEC System. There were 433 sputum samples with AFB positive collected from MDR PTB patients. Resistance to RHES was found in 22 (14%) AFB +1, 19 (15%) AFB +2, and 29 (20%) AFB +3. Resistance to RHS was found in 22 (14%) AFB +1, 12 (9%) AFB +2, and 13 (9%) AFB +3. Resistance to RHE was found in 39 (25%) AFB +1, 38 (29%) AFB +2, and 35 (24%) AFB +3. Resistance to RH was found in 74 (47%) AFB +1, 61 (47%) AFB +2, and 69 (47%) AFB +3. Statistic analysis by Spearman test showed that there was no significant correlation between AFB gradation and first-line anti-TB drug resistance patterns. Acquired resistance to RHES can also found in lower bacillary load AFB +1.

**Keywords:** MDR pulmonary TB, AFB grading, first line anti-TB drug resistance pattern

#### ABSTRAK

Tuberkulosis multidrug-resistan (TB-MDR) merupakan salah satu masalah kesehatan utama di dunia. Pemeriksaan basil tahan asam (BTA) pada sampel dahak merupakan komponen yang penting dalam diagnosis dan pemantauan hasil pengobatan pasien TB paru. Pasien TB paru dengan jumlah BTA yang lebih tinggi memiliki potensi tinggi terjadi resistensi obat. Pasien dengan jumlah BTA yang lebih tinggi menunjukkan jumlah basil yang lebih banyak dan memiliki potensi terjadi resistensi yang lebih tinggi. Penelitian ini bertujuan untuk mengevaluasi hubungan antara gradasi BTA dan pola resistensi obat anti-TB lini pertama pada pasien TB paru MDR. Studi ini merupakan studi retrospektif yang dilakukan di Rumah Sakit Dr. Soetomo pada bulan Agustus 2009 hingga bulan April 2018. Sampel dahak diambil dari pasien TB paru MDR. Pemeriksaan dahak dilakukan menggunakan pewarnaan Ziehl–Neelsen dan jumlah BTA diukur sesuai dengan kriteria IUATLD. Sampel BTA positif dilakukan evaluasi pola resistensi obat anti-TB lini pertama berdasarkan uji kultur dan resistensi dengan Sistem BACTEC MGIT 960. Terdapat 433 sampel dahak dengan BTA positif dari pasien TB paru MDR. Resistensi terhadap RHES ditemukan pada 22 (14%) BTA +1, 12 (9%) BTA +2, dan 13 (9%) BTA +3. Resistensi terhadap RHE ditemukan pada 39 (25%) BTA +1, 38 (29%) BTA +2, dan 35 (24%) BTA +3. Resistensi terhadap RH ditemukan pada 74 (47%) BTA +1, 61 (47%) BTA +2, dan 69 (47%) BTA +3. Analisis statistik dengan uji Spearman menunjukkan bahwa tidak terdapat

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hubungan yang signifikan antara gradasi BTA dan pola resistensi obat anti-TB lini pertama. Pola resistensi RHES juga dapat ditemukan pada jumlah basil yang lebih rendah BTA +1.

**Kata kunci:** TB paru MDR, gradasi BTA, pola resistensi obat anti-TB lini pertama

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## INTRODUCTION

Drug-resistant tuberculosis (DR TB) continues to be a public health crisis. In 2017, around 558,000 people in the world developed rifampicin-resistant TB (RR-TB) and 82% had multidrug-resistant TB (MDR-TB).<sup>1</sup> MDR-TB is defined as TB which caused by strain *Mycobacterium tuberculosis* resistant at least to isoniazid (H) and rifampicin (R), two of the main first-line anti-TB drugs.<sup>2</sup> First-line anti-TB drugs consist of isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E), and streptomycin (S). Globally, Indonesia is the 7<sup>th</sup> rank in the estimated incidence of RR-TB cases in 2017 is 23.000 people with MDR percentage among RR-TB cases was 91%.<sup>1</sup>

From all of TB cases, 2.4% of new TB cases and 13% of previously treated cases had MDR/RR-TB. This means the miss management of TB cases is still dominant as the cause of DR TB. Drug resistance occurs when drug-susceptible TB (DS TB) patients receive inadequately or interrupted therapy which leads to the selection of drug-resistant bacteria and ‘acquired’ drug resistance. Infectious patients who are infected by resistant strain *Mycobacterium tuberculosis* could spread through airborne droplets as transmitted drug resistance.<sup>3</sup>

Acid-fast bacilli (AFB) microscopy examination is a common simple tool for the diagnosis and treatment outcome monitoring of pulmonary TB.<sup>4</sup> Patients with higher AFB grade indicates higher bacillary load and increasing baseline drug resistance had higher rates of acquired resistance.<sup>5</sup> The recent dogma stated that the level of resistance to INH and RIF (required for MDR-TB) was caused by the individual mutation rates for INH and RIF; that is, in the order of

10<sup>-6</sup>. For the evolution of MDR strains, a total population of at least 10<sup>6</sup> bacilli must be present in each infected person.<sup>6</sup> The possibility that a single drug-resistant mutant may arise earlier after infection, and could replicate to a large enough population from which the possibility of a second drug-resistance mutation will not be too slow.<sup>7</sup> The potential drug-resistant mutation is varied in each drug, ranging from around 1 in 10<sup>8</sup> bacilli for rifampicin, to about 1 in 10<sup>6</sup> bacilli for isoniazid, streptomycin, and ethambutol. Besides, *Mycobacterium tuberculosis* consists of various phylogenetic lineages,<sup>8</sup> that could have some intrinsic drug resistance character in the bacilli population of the PTB patients. On the other hand, MDR-PTB cases with several an active disease process with AFB bacilli production in sputum with many population characteristics of anti-TB resistance that related to multi factors.

Some clinicians assume that more amount of AFB can cause acquired more drug resistance. This study aims to determine the drug resistance pattern of all positive smear in MDR PTB patients and evaluate its correlation with AFB microscopy grading.

## MATERIALS AND METHODS

### Study Definition

Patients were divided by a history of previous TB treatment according to WHO guideline<sup>9</sup>

1. New cases: who have never been treated for TB or have taken anti-TB drugs for less than 1 month.
2. Previously treated patients have received 1 month or more of anti-TB drugs in the past. They are further classified by the outcome of their most recent course of treatment:

- a. Relapse patients have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by re-infection).
- b. Treatment after failure: patients are those who have previously been treated for TB and whose treatment failed at the end of their most recent course of treatment (WHO category I regimen or WHO category II regimen).
  - WHO category I regimen: 2 (HRZE)/ 4(HR)3 or 4(HR)
  - WHO category II regimen: 2 (HRZE)S/ (HRZE)/ 5(HR) 3E3 or 5(HR)E
- c. Treatment after loss to follow-up: patients have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment (these were previously known as a treatment after default patients).
- d. Other previously treated patients are those who have previously been treated for TB but whose outcome after their most recent course of treatment is unknown or undocumented.

### Study Subjects and Design

This was a retrospective study. Samples were collected from all MDR pulmonary TB (MDR PTB) patients who are treated from August 2009 to April 2018 in Dr. Soetomo Hospital. The medical records of enrolled patients were reviewed to obtain their microbiological examinations. Sputum samples were taken from new and previously treated MDR PTB patients. Sputum smear examination was done using Ziehl–Neelsen staining. Direct smears were made from each sputum sample and were stained with Ziehl-Neelsen (ZN) stain according to the WHO recommendation. AFBs identified were graded according to the International Union against Tuberculosis and Lung Disease (IUATLD) and the WHO smear grading scale. Findings were

scored as follows: 1–9 AFB/100 fields (1+); 1–9 AFB/10 fields (2+); and 1–9 AFB/ field (3+). Each slide was examined by three independent readers to ascertain the presence of AFB and grade positive smears. The slide readers were blinded on the clinical and laboratory diagnoses of the participants whose samples were studied. Samples with positive smear were evaluated for resistance pattern based on culture method using MGIT 960 BACTEC System for determinate the sensitivity to Rifampicin (R), Isoniazid (H), Ethambutol (E), and Streptomycin (S). Examination of microscopic sputum smears, culture method for identification and drug sensitivity test were carried out at the Surabaya Health Laboratory Center which has been certified by WHO. Statistic analysis using the Spearman test was used to analyze the significance of AFB grading and resistance pattern.

### RESULTS AND DISCUSSION

There were 433 MDR-TB patients with positive smear, 253 (58.4%) men and 180 (41.6%) women in MDR-TB clinic care of Dr. Soetomo Hospital from August 2009 to April 2018. The number of MDR PTB patients were higher in men than women in this study with 253 (58.4%) and 180 (41.6%) women, respectively. Another study also found that the MDR/RR TB strains were three times more common in men than women.<sup>10</sup> Being a man or woman can be a factor to develop drug resistance; however, the findings vary on the subject. A global prevalence study did not find sex to be a risk factor for MDR-TB.<sup>11</sup>

The average age of MDR PTB patients was 43.82 years old and most MDR TB patients were productive with age range 15-49 year-old with a count of 291 (67.2%). Globally, there were cases in all countries and age groups but overall 90% were adults (aged  $\geq 15$  years).<sup>1</sup> A study in Switzerland reported that age  $< 35$  years old increased risk of resistance to first-line drugs (OR=1.5; 95% CI 1.0–2.3).<sup>12</sup>

Based on TB treatment history, MDR PTB patients were divided into new cases and previously treated cases (relapse, return after

default, failure of the WHO category I, failure of the WHO category II, and other cases such as unstandardized treatment). Most of MDR PTB patients were ones with previously treated with 426 (98%). Relapse cases were dominant with 160 (36.9%), followed by failures of the WHO Category I regimen with 110 (25.4%), and return after default with 91 (21%). This result was shown in Table 1.

There were 426 (98%) of MDR-TB patients were coming from patients with the previous history of TB treatment in this study. Previously treated TB patients were a risk factor for MDR-TB.<sup>13</sup> Previous anti-TB treatment was by far a solid predictor of drug resistance.<sup>14</sup> Previously treated TB patients had a higher chance as many as 8.1 times to develop an MDR-TB infection compared to newly diagnosed TB patients.<sup>15</sup> In this study, relapse cases were the most common with 160 cases (36.9%), followed by failures of the WHO Category I regimen with 110 cases (25.4%). Relapse cases were dominant among patients with MDR-TB in this study. The previous study reported that most of drug-resistant TB were relapse cases with 123/290 patients (42.4%), followed by treatment failures with 123/290 (34.8%).<sup>16</sup> The dominance of relapse cases among MDR-TB patients may caused by inadequate treatment and less compliance of patient during previous treatment resulted dormant MDR-TB. Subsequently, the survival of dormant MDR-TB increased the risk of TB relapse.<sup>17</sup> The dominance of relapse cases also happened because TB recurrence resulted from either relapse or reinfection was remained defined as relapse according to the WHO guideline. To defined relapse or reinfection cases, the

examination of *Mycobacterium tuberculosis* strain was needed to know whether it was relapse of an original infection or exogenous reinfection with a new *Mycobacterium tuberculosis* strain. In the previous study, 51.4% of relapse happened in  $\leq 2$  years and 48.6% of relapse happened in  $> 2$  years, while 57.1% of reinfection happened in  $> 2$  years and 42.9% reinfection happened in  $\leq 2$  years.<sup>17</sup>

Although new TB diagnosing technologies have been improved, the use of AFB microscopy still the main of the diagnostic<sup>18</sup> and patients with positive AFB are often considered as MDR-TB due to greater AFB leads the bacterial mutation. Patients with higher bacterial load are more potential for drug-resistant mutations and have a greater risk of developing MDR-TB.<sup>19</sup> Initial AFB sputum smear  $\geq 3+$  was correlated with acquired drug resistance.<sup>5</sup> Of the 433 sputum samples with AFB positive collected from MDR PTB patients, resistance to RHES was 14% in AFB +1, 15% in AFB +2, and 20% in AFB +3. Resistance to RHS was 14% in AFB +1, 9% in AFB +2, and 9% in AFB +3. Resistance to RHE was 25% in AFB +1, 29% in AFB +2, and 24% in AFB +3. Resistance to RH was 47% in AFB +1, 47% in AFB +2, and 47% in AFB +3. Based on statistic analysis by Spearman test, there was no significant correlation between AFB gradation and resistance pattern with p-value 0.786 as presented in Table 2.

The results in Table 2 showed that resistance to more drugs was also happened by the lower AFB grading (AFB +1) and indicated that the grade of AFB might not represented the number of *Mycobacterium tuberculosis*. AFB-positive smears may be because of the presence of

**Table 1.** History of TB treatment profile of MDR TB patients in Dr. Soetomo Hospital.

Variable	R+H	R+H+E	R+H+S	R+H+E+S	Total
New cases	3 (43%)	3 (43%)	0 (0%)	1 (14%)	7
Previously treated cases	201 (47%)	109 (26%)	47 (11%)	69 (16%)	426
• Failure treatment with WHO Category II regimen	19 (34.5%)	16 (29%)	8 (14.5%)	12 (22%)	55
• Failure treatment with WHO Category I regimen	53 (48%)	29 (26%)	8 (7%)	20 (18%)	110
• Relapse	84 (52.5%)	39 (24%)	17 (11%)	20 (12.5%)	160
• Return after default	43 (47%)	22 (24%)	14 (15%)	12 (13%)	91
• Other case	2 (20%)	3 (30%)	0 (0%)	5 (50%)	10

**Table 2.** Analysis of correlation between AFB grading and the first line anti-TB drug resistance pattern.

AFB Grading	Resistance Pattern				Total	P Value
	R+H+E+S	R+H+S	R+H+E	R+H		
+++	29 (20%)	13 ( 9%)	35 (24%)	69 (47%)	<b>146 ( 34%)</b>	0.786
++	19 (15%)	12 ( 9%)	38 (29%)	61 (47%)		
+	22 (14%)	22 (14%)	39 (25%)	74 (47%)		
Total	70 (16%)	47 (11%)	112 (26%)	204 (47%)		

\*P value based on Spearman Test. Correlation coefficient (0.013).

**Table 3.** Correlation between AFB grading vs. every treated group.

History of TB treatment	AFB			P-value
	+	++	+++	
New cases (n=7)	2 (28.5%)	2 (28.5%)	3 (43%)	0.895
Failure treatment with WHO Category II regimen (n=55)	18 (32.7%)	18 (32.7%)	19 (34.6%)	
Failure treatment with WHO Category I regimen (n=110)	43 (39%)	31 (28%)	36 (33%)	
Relapse (n=160)	53 (33%)	49 (31%)	58 (36%)	
Return after default (n=91)	37 (40%)	27 (30%)	27 (30%)	
Other case (n=10)	4 (40%)	3 (30%)	3 (30%)	

nonviable *Mycobacterium tuberculosis* bacilli or nontuberculous mycobacteria (NTM).<sup>20</sup>

Our study found that the AFB grading did not represent the resistance pattern of first-line anti-TB drugs. AFB +1, which was the lower bacillary load, also showed resistance to RHES. Based on statistical analysis using the Spearman test, AFB grading was not correlated with the resistance pattern of MDR TB patients with  $p$  0.786. This result showed that the bacillary load did not affect the resistance to some TB drugs. A different result was shown by another study that reported higher smear grade (+2 and +3) has a higher rate of MDR-TB/ RIF resistance with 76/256 (29.7%) compared with smear grades of +1, scanty positive and negative with 61/301 (20.3%) ( $p$ -value = 0.01).<sup>10</sup> There was no reveal the correlation of the first-line anti-TB drug resistance pattern with AFB grading in this study. Resistance to more drugs (RHES) also found in patients with AFB +1.

Analysis of correlation between AFB grading and every treated group showed that there was not a significant difference with a  $p$ -value of 0.895 as presented in Table 3. The definition of each group has been described in the methodology.

The results in Table 3 showed that the AFB grading was not affected by the history of TB treatment. Actually, AFB smear can be used to assess TB treatment outcome, but careful examination of microbiologic status, including culture and drug susceptibility testing were also needed to confirm the AFB smear examination.<sup>4</sup>

Greater AFB grading is often considered associated with the incidence of drug resistance. A higher AFB grading represented higher bacilli and it possible to acquired drug resistance. Acquired resistance to rifampicin was estimated by mutation of  $10^8$  bacilli and acquired resistance to isoniazid, streptomycin, and ethambutol by mutation of  $10^6$  bacilli.<sup>21</sup> This rate might also be affected by the drug concentration in the medium, the drug resistance profile of the strain and its genetic background.<sup>22</sup> Drug resistance-associated genes were *katG* and *inhA* in isoniazid, *rpoB* in rifampicin, *rpsL* in streptomycin, and *embB* in ethambutol.<sup>23</sup> Previous studies reported that there were varies drug resistance patterns among sputum-smears positive; MDR-TB, non-MDR two drug resistance, and resistance to any one of the first line of drugs (isoniazid, ethambutol, and rifampicin).<sup>24</sup>

Acquired resistance to more drugs may correlate with *Mycobacterium tuberculosis* strain in MDR pulmonary TB patients. Different strain of *Mycobacterium tuberculosis* also represented different frequencies of genes which played role in drug resistance. The prevalence of specific drug resistance-associated mutations also varies within the lineage, such as the frequencies of the *rpoB* S531L and *katG* S315T mutations are greater in the modern (typical) Beijing strains than in ancient (atypical) ones. There was a significant variation in the mutation rates of strains, the study also showed that strains from Lineage 2 of *Mycobacterium tuberculosis* (includes Beijing family of strains) acquire drug resistance *in vitro* rapidly than strains from Lineage 4.<sup>22,25</sup>

## CONCLUSIONS

There was no significant correlation between the first-line anti-TB resistance pattern of MDR PTB strain with AFB microscopy grading. Acquired resistance to RHES can also found in lower bacillary load AFB +1.

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## CONFLICT OF INTEREST

There is no conflict of interest of this paper.

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