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Research Report

## ACID-FAST BACILLI CONVERSION OF *BEIJING* AND NON-*BEIJING* STRAIN OF PULMONARY TUBERCULOSIS IN SOUTH SULAWESI

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### ABSTRACT

*Beijing* strains are a major part of the *Mycobacterium tuberculosis* Asian phylogenetic lineage. *Beijing* strains represent about 50% of all TB strains in East Asia and at least 13% of strains worldwide. *Beijing* strain of *Mycobacterium tuberculosis* is presumed as the factor of the increase in bacteria virulence and drug resistance, and the contributor in treatment failure. The aim of this study was to analyze the association between acid-fast bacilli conversion with strain genotype *Beijing* and non-*Beijing* of pulmonary tuberculosis in South Sulawesi. The design of research was observational analytic with prospective approach. The sampling technique used consecutive sampling. Data were taken from active pulmonary tuberculosis patients' medical record in Balai Besar Kesehatan Paru Masyarakat Makassar (Pulmonary Health Center of Makassar) and Community Health Center in Gowa Regency, South Sulawesi from March to June 2018. Collected sputum samples were screened for AFB and identified as *Beijing* strain and non *Beijing* strains using Multiplex PCR in Tropical Disease Institute of Universitas Airlangga. The results is showed that the characteristics of the respondents consisted of 12 respondents (33.3%) aged 56-65 years, 25 respondents (69.4%) men and 28 respondents (77.8%) had low category gradation of AFB smear. Univariate analysis showed 6 respondents (16.7%) with *Beijing* strains, 30 respondents (83.3%) with non-*Beijing* strains, 32 respondents (88.9%) conversion sputum AFB and 4 respondents (11.1%) non conversion sputum AFB. Bivariate analysis with Chi-Square statistical test shows that  $p$  value  $0.022 < 0,05$ , that means there was association of *Beijing* strains with BTA conversion. Microscopic examination of BTA can be used to monitor and evaluate the treatment of new pulmonary TB patients undergoing treatment and the *Beijing* *Mycobacterium tuberculosis* strain has a significant correlation with the treatment failure of anti-tuberculosis drugs in South Sulawesi.

**Keywords:** AFB conversion, *Beijing* strain, *Mycobacterium tuberculosis*, pulmonary tuberculosis, Treatment failure

### ABSTRAK

Strain *Beijing* merupakan bagian utama dari garis keturunan filogenetik *Mycobacterium tuberculosis* Asia. Strain *Beijing* mewakili sekitar 50% dari seluruh strain TB di Asia Timur dan setidaknya 13% dari strain seluruh dunia. Strain *Beijing* dari *Mycobacterium tuberculosis* diprediksi penyebab peningkatan virulensi bakteri, resistensi terhadap pengobatan, dan berkontribusi dalam kegagalan pengobatan. Penelitian ini bertujuan menganalisis asosiasi konversi BTA mikroskopis dengan strain *Beijing* dan non *Beijing* pada pasien tuberculosis baru di Sulawesi Selatan. Jenis penelitian adalah analitik observasional dengan rancangan penelitian prospektif. Teknik pengambilan sampel menggunakan Konsektif sampling. Data diperoleh dari rekam medis pasien dengan TB paru aktif yang dirawat di Balai Besar Kesehatan Paru Masyarakat Makassar dan Puskesmas di Kabupaten Gowa, Sulawesi Selatan pada Maret sampai dengan Juli 2018. Sampel sputum yang dikoleksi dilakukan pemeriksaan BTA kemudian diidentifikasi strain *Beijing* dan non *Beijing* dengan metode Multiplex PCR di Institute penyakit tropis Universitas Airlangga. Hasil penelitian menunjukkan karakteristik responden terdiri dari 12 responden (33,3%) berusia 56-65 tahun, 25 responden (69,4%) laki-laki dan 28 responden (77,8%) memiliki

gradasi BTA kategori Low. Analisis univariat menunjukkan 6 responden (16,7%) dengan strain Beijing, 30 responden (83,3%) dengan strain non-Beijing, 32 responden (88,9%) mengalami konversi sputum BTA dan 4 responden (11,1 % tidak mengalami konversi sputum BTA). Analisis bivariat dengan uji statistik Chi-Square menunjukkan  $p$  value 0,022, terdapat asosiasi strain Beijing dengan konversi BTA. Pemeriksaan BTA dengan mikroskopis dapat digunakan untuk monitoring dan evaluasi pengobatan pasien TB Paru yang baru menjalani pengobatan serta strain Beijing *Mycobacterium tuberculosis* memiliki korelasi yang signifikan dengan kegagalan pengobatan obat anti tuberculosis di Sulawesi Selatan.

**Kata Kunci:** Konversi BTA, Strain Beijing, *Mycobacterium Tuberculosis*, TBC Paru, Kegagalan Pengobatan

## INTRODUCTION

Pulmonary tuberculosis (TB) is both an acute and chronic disease which caused by *Mycobacterium tuberculosis* that attacks the lungs. The disease spreads from one individual to another through the respiratory system. In 2015, it was estimated that there were 10.4 million new cases of TB worldwide; of which 5.9 million are found in adult male, 3.5 million in adult female, and 1 million in children. Some countries in South and East Asia such as The Democratic Republic of Korea, Indonesia, Myanmar, Bangladesh, India, and Thailand comprise 46.5% of the total global number. India and Indonesia alone make up one-third of the worldwide TB number, each having 23% and 10% respectively<sup>1</sup>.

Global efforts on TB control were required integrated and coordinated responses to monitor and assess global outbreak of TB, drug resistance surveillance, and high-risk population. An integrated approach is a must, combining networks of laboratories, TB control programs, and application of state-of-the-art molecular techniques to identify and report tuberculosis bacilli clones occurring around the world<sup>2</sup>.

Tuberculosis occurrence in Indonesia is estimated at 1,020,000 cases, with only 330,729 of those are reported. Of all the cases in Indonesia, 93% are pulmonary TB with 64% of those are diagnosed through microscopic evaluation of acid-fast bacilli smear. The large number of untreated TB will become the source of further infections, creating urgency in having continuous preventive and curative measures. The success of a TB countermeasure program is linked to the success in DOTS treatment. Such treatment is deemed to be a success if it is evidenced by sputum AFB conversion at the final phase of the intensive treatment (2 months) reaching at least 80% and recovery rate at the end of treatment reaching more than 85%<sup>3</sup>. Acid Fact Bacilli conversion is a strong predictor and the initial success of therapy in pulmonary TB<sup>4</sup>. Sputum conversion in pulmonary TB was determined based on the absence of Acid Fact Bacilli (AFB) in sputum taken at the end of the 2nd month of treatment. Sputum conversion in pulmonary TB cases occurred at the end of the first month (60-80%) and at the end of the second month (95%)<sup>4</sup>.

AFB conversion is one of the methods used in Indonesia which have high accuracy, fast and economical costs. Another method was Chest X Ray but the results are not specific to diagnosis tuberculosis<sup>5</sup>. In addition there are

culture methods that have a higher sensitivity but the cost not effective and needed long time for know the result of diagnosis. The research of Wang X, et al was showed that by using direct smear method Ziehl Nielsen staining obtained a sensitivity of 40%, then in the same sample culture method was carried out so the sensitivity value would increase by 65%<sup>6</sup>.

Sputum AFB conversion can be affected by previous medical history. *Mycobacterium tuberculosis* exposed with antibiotics might undergone genetic mutation as a result of its biological trait to defend itself. Mutation might occur to a specific gene targeted by the medication, creating risk of drug resistance, which in turn resulting in sputum AFB conversion failure<sup>7</sup>.

Another factor correlating with sputum AFB conversion is the *Mycobacterium tuberculosis*' genotype. The genotype of *Mycobacterium tuberculosis* that attack TB patients in Indonesia are different from one province to another as a result of the geographical differences of each province<sup>8</sup>. Different strain has different characteristics; one of which is the tendency to develop antibiotics resistance. *Mycobacterium tuberculosis complex* (MTBC) strains which are known to be easier to treat are *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium microti*, *Mycobacterium canneti* and *M. tuberculosis*<sup>9</sup>.

Molecular epidemiology studies have pointed out that *Mycobacterium tuberculosis*' genotype is varied based on geographical distribution<sup>10</sup>. Some pathogen hosts have undergone adjustments while specific genotype has evolved to increase its pathogenicity and virulence. In some East Asian countries such as China and Hong Kong, 86% of the occurring genotype is *Beijing* strain<sup>11</sup>. In contrast, Indonesia has more varied distributions. South Sulawesi, for instance, is still dominated by *Beijing* strain. *Beijing* strain genotypes have specific characteristic such as contribute to the spread of drug-resistant TB and clinically related to treatment failure<sup>12</sup>. Thai studies is showed infection with *Beijing* genotype is a significant risk factor for bacterial persistence in treatment that results in treatment failure or relapse inside 2 years<sup>13</sup>.

Lisdawati's research on *Beijing* strain distribution in Indonesia is showed that in Palembang and Lampung, *Beijing* strain occurrence number is 31.48%; Serang, Jakarta, Bandung, and Surabaya have 28.83%; Banjarmasin and Pontianak have 16.18%; and Makassar has 25.93%. *Beijing* strain distribution in male is 24.2%, while in female is 15.3%. Specifically in Makassar, other occurring

strains are sub-type EAI1 (1 pattern), H1 (1 pattern), LAM (2 patterns), EA12 (2 patterns), U (3 patterns), UlikeS (1 pattern), EA15 (1 pattern), Ulikely LAM (1 pattern), MANU2 (1 pattern), and H3 (2 patterns). The sample also holds 1 isolate which was showed pattern of *M. bovis*<sup>14</sup>.

Identifying *Beijing* genotype can be done through various methods, such as spoligotyping, IS6110 RFLP, PCR, and sequencing<sup>15</sup>. These methods focus on various types of protein in specific target genes. Nikajima's research points out that *Mycobacterium tuberculosis genome H37Rv* is able to identify *Beijing* strain, specifically the Rv0679c protein with the size of 163bp<sup>15</sup>. Data from South Sulawesi Provincial Health Office in the year 2016 shows that there were 13,659 TB cases with 12,965 cases of TB (7,180), 97 cases of MDR TB, and 597 cases of TB in children. In the cases of AFB positive TB, treatment success rate was 86%, less than the targeted 90%. Beside that, success rate just 82,5% (target more > 90%)<sup>16</sup>.

## METHOD

This is an analytical observational study with cross-sectional analysis design. The research was done in the City of Makassar and Gowa Regency from March to June 2018. The samples used were 36 new patients diagnosed with pulmonary TB, with an age of 15 to 65 years. Ethical clearance was issued by Ethical Committee of Universitas Airlangga numbered 82/EC/KEPK/FKUA/2018. Tuberculosis patients were diagnosed using Zielh Neelsen AFB smear method. Conversion occurred if there was a change in the result of AFB smear: from positive to negative during the 2-month treatment. Detection of *Beijing* strain was done by decontamination using Petroff alkali method and extraction of DNA using Qiagen kits. Amplification of *Beijing* strain was done with Multiplex PCR. The PCR Mix had a total volume of 20 µl which were comprised of 10 µl of PCR Buffer, 1 µl of ON-1002 (FW), ON-1258 (R1)<sup>α</sup>, and ON-1127 (R2) primers, 6 µl of DW, and 2 µl of template DNA. The next step was a 25-cycle of pre-denaturation at 95°C for 1 minute, denaturation at 95°C for 10 seconds, annealing at 66°C for 10 seconds, and extension at 72°C for 15 seconds. Upon finishing, a final extension was done at 72°C for 3 minutes and an elongation at 15°C for 5 minutes<sup>16</sup>. Data analysis was performed using SPSS 17. Correlation between sputum AFB conversion with *Beijing* strain was assessed using bivariate analysis by Chi-square test.

## RESULT

A preliminary survey was performed to collect data of respondents' characteristics such as age, sex, and AFB gradation. The results are presented in the Table 1:

*Mycobacterium tuberculosis* was isolated from 50 patients are AFB positive, but only 36 fit the criteria. *Beijing* strain is identified through Multiplex PCR with the result showing 6 samples (16.7%) are *Beijing* strain and 30 samples (83.3%) are non-*Beijing*. Treatment success rate is identified by microscopic examination after 2 months of treatment. As much as 32 patients (88.9%) are found to have undergone conversion, while the other 4 (11.1%) are not. The results are presented in the Table 2.

Chi-square test yields p-value of 0.010 < 0.05, meaning that there is a significant correlation between *Beijing* strain genotype with sputum AFB conversion. The results are presented in the Table 3.

**Table 1.** Respondent distribution based on age, sex, and AFB gradation (N=36)

No	Characteristic	Frequency (n)	Percentage (%)
1	Age (years old)	16-25	16.7
		26-35	13.9
		36-45	13.9
		46-55	22.2
		56-65	33.3
2	Sex	Male	69.4
		Female	30.6
3	AFB Gradation	Low	77.8
		Medium	8.3
		High	13.9

**Table 2.** Frequency distribution of genotype strain and AFB smear conversion

No	Research variables	Frequency (n)	Percentage (%)
1	Genotype	<i>Beijing</i> Strain	16.7
		non- <i>Beijing</i> Strain	83.3
2	AFB Conversion	Conversion	88.9
		Non-conversion	11.1

**Table 3.** Correlation between genotype and sputum AFB conversion

MTB Genotype	AFB Conversion				Total	p-value	OR
	Non-conversion		Conversion				
	N	%	N	%			
Beijing	3	75.0	3	9.4	6	16.7	0.010 15.0 (95%, CI 1.8-120.8)
Non- <i>Beijing</i>	1	25.0	29	90.6	30	83.3	
Total (n)	4	11.1	32	89.9	36	100	

## DISCUSSION

This research was showed that AFB conversion rate of new patients with AFB positive is 89.9%. According to WHO, the conversion rate must be more than 80%<sup>17</sup>. Diagnosis and conversion are performed with AFB method using microscopes. Examination on two sputum specimens is sufficient to identify the majority (95-98%) of AFB-positive TB patients. the policy of WHO recommend to using two spesimen sputum on case finding using microscopy to get setting with appropriate external quality assessment and high-quality documentation. The microscopy of both specimens must be screened. In settings with appropriate quality assurance procedure, a case is defined as a person with one positive smear—meaning at least 1 AFB in 100 microscopic fields<sup>18</sup>. In 2010, WHO confirmed the accuracy of microscopy of two consecutive sputum specimens on the same day for TB diagnosis, allowing treatment to take place since the first visit to a health center facility<sup>17</sup>.

Microscopy benefits in terms of simplicity and cost efficiency, allowing immediate detection of the most infectious pulmonary TB. Sputum specimen from patients with pulmonary TB, especially those having cavity diseases, often have enough bacteria to be detected using microscopes. Microscopy is also suitable for periphery-level laboratories, or higher. It can be performed safely even in laboratories with low alert value, reducing the risk of TB infection in laboratories. It is a simple, speedy, and inexpensive test needed as a follow up measure for patients with high risk of TB. Despite the benefits, microscopy also has limitation for AFB. It is unable to distinguish *Mycobacterium tuberculosis complex* from non-tuberculous mycobacteria. It cannot tell the difference between organisms that should be exterminated from those that actually pose no threat. It also cannot differentiate drug-sensitive strains from the resistant ones<sup>18</sup>.

Sputum AFB conversion in pulmonary TB patients takes place after intensive phase of the treatment. AFB conversion is the change in sputum smear from AFB positive to AFB negative after undergoing intensive treatment<sup>19</sup>. Based on Table 2, it can be seen that AFB conversion occurs in 32 samples (88.9%) and does not occur in 4 samples (11.1%). This research was showed non conversion rate 11,1 %, based on interview with health worker in the Public Health Centre, the pasien who non conversion after 2 month treatment have comorbid diseases such as Diabetes Melitus. Garrido research was showed that there was relationship between Diabetes Melitus with treatment failure<sup>20</sup>. This research was showed a conversion rate of 88.9%, fitting the indicator from the Ministry of Health of >80%. Conversion acts as an indicator of treatment success. Success of a treatment is also influenced by age, sex, medical history, presence of antibiotic resistance and bilateral radiological lesion<sup>21, 22</sup>. Smear acid bacilli, retreatment, comorbid diseases, and education<sup>23</sup>.

Beijing genotype strains have specific properties such as the effects of the BCG vaccine, increased virulence, and risk

of treatment failure. clinically, there were no differences in signs and symptoms of the Beijing and non-Beijing genotype strains<sup>24</sup>, but Parwati research is showed that the proportion of patients in the Beijing strain who had fever symptoms was higher than the strain non Beijing<sup>25</sup>. At the molecular level, Beijing strains have specific properties in terms of protein and lipid structure and their interaction with the human defense system. Finally, the Beijing genotype has polymorphisms in immune genes, showed the co-evolutionary of human-mycobacteria. The emergence of the Beijing genotype family may represent the response of M tuberculosis to vaccination or antibiotic treatment, with a negative reaction to tuberculosis control<sup>26</sup>.

The findings are showed that the result of sputum Multiplex PCR of the respondents mostly (30 samples, or 83.3%) are non-Beijing strain and only a few (6 samples, or 16.7%) are Beijing strain. Research done by Octavian using spoligotyping shows that there are 8% Beijing strain in Papua<sup>19</sup>. Research by Lisdawati points out a proportion of Beijing strain in Makassar to be as much as 25.93%<sup>13</sup>. According to researcher's assumption, the percentage of Beijing in Makassar is quite high compared to other regions in the Eastern Region of Indonesia, Makassar society has a higher level of mobilization and geographically located in Central Indonesia making it easier to move to other area. Data analysis using Chi-square test yields p-value of 0.022 < 0.05, meaning that there is a significant correlation between Beijing strain genotype and sputum AFB conversion. This finding is also advocated by Parwati, claiming that there is a correlation between Beijing strain and treatment failure<sup>26</sup>. This research is different from Hang's which focuses on Beijing strain's sublineage of modern, ancient, and non-Beijing. Hang's research also shows that there is no correlation between the strain of *Mycobacterium tuberculosis* and treatment failure<sup>27</sup>. A study examining clinical pathways and treatment outcomes depends on the genotype of the Mycobacterium (MTB) strain among various age groups of TB patients. This study was conducted on 6 strains including Beijing, LAM, Haarlem, Ural / Uganda I, S, Africanum and showed that Beijing was included in the group with a low treatment success rate<sup>28</sup>. Mourik's research is showed that there is still a presence of active bacteria in the first two months of treatment on test animals infected with Mycobacterium tuberculosis Beijing strain<sup>29</sup>. Beijing strains was also associated with the incidence of MDR and recurrence of TB patients. Vyazovaya's research was showed mutations in rpoB531 were most commonly found among Beijing isolates (60 of 72 isolates with rpoB mutations), five isolates (four of which were Beijing) were showed multiple or triple mutations in rpoB. INH resistance mutations, the katG315 mutation is also the most common in Beijing isolates<sup>30</sup>. Studies from Estonia, China, Korea and Taiwan were showed a significantly higher MDR frequency among Beijing descendants<sup>31</sup>. A study conducted in Sweden which identified 13% of Beijing strains for 15 years. The proportion of strains with MDR was significantly higher among Beijing strains than in non-Beijing strains<sup>31</sup>.

Different with Rutaihua's research, the existence of Beijing strains in Africa is not related to anti-tuberculosis drug resistance<sup>32</sup>. This study was supported Soolingen's study of the relationship between genotype and resistance between unclear treatment failure<sup>33</sup>.

## CONCLUSION

Although it is true that AFB microscopy is vital in monitoring and evaluating one-line anti-tuberculosis treatment in pulmonary TB patients in TB endemic areas, early care management for pulmonary TB patients also needs to consider the lineage of the *Mycobacterium tuberculosis* strain, e.g. *Beijing* strain. At the molecular level, Beijing strains have specific properties in terms of protein and lipid structure and their interaction with the human immune system. This becomes imperative given the fact that the strain of *Mycobacterium tuberculosis* is a determinant of treatment success of the patients and examine the implications for future strategies.

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