



DETECTION OF THE RESISTANCE OF *MYCOBACTERIUM TUBERCULOSIS* FROM SPECIMENS WITH TB PATIENTS IN SEMARANG BALKESMAS

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Abstract

The government has worked hard in an effort to eradicate tuberculosis by forming a program Temukan Obati Sampai Sembuh Tuberculosis (TOSS TBC) to free the Indonesian people from Tuberculosis in 2025. Directly Observed Treatment Short-course (DOTS) strategy treatment is the best effort in treating TB, but other problems arise related to the treatment, one of which is the emergence of resistance to Obat Anti Tuberculosis (OAT). Detection of the occurrence of resistance of *Mycobacterium tuberculosis* from sputum specimens of tuberculosis patients in BALKESMAS Semarang used a non-experimental cross-sectional design with a non-intervention approach survey method. Sputum samples were from TB patients who did not convert during treatment. Percentage of culture results from 9 patients was 33.3. DST test from sensitive first-line OAT samples, morphological and size changes (1.82 - 1.87 μm) shows that first-line drugs were still effective for treatment inpatients who took part in this study. Samples conducted as a study can still be treated with first-line drugs. Patients must be obedient to take medication for speed healing

Keywords: *Mycobacterium tuberculosis*, not conversion, resistance

1. Introduction

Tuberculosis cases in Central Java in 2017 were noted 42,272 cases out of a total population of 34,257,865 with positive Acid-fast bacilli (AFB) cases of 18,248 (10,614 men and 7,734 women). The number of Case notification rates (CNR) was 123 and the number of Positive AFB was 53. Positive pulmonary tuberculosis coverage had cured 11,758 or 93.07% -- 876 or 6.93% with complete treatment. The treatment success was 12,634 or 72.84% (Ministry of Health, RI, 2018).

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strategy treatment is the best effort in treating TB, but other problems arise related to treatment, one of which is the emergence of resistance to Obat Anti Tuberculosis (OAT).

The standard of treatment can be achieved with about 98% recovery and less than 5% relapse if Isoniazid (INH), rifampicin (RMP), Prazinamide (PZA) and Entambutol (EMB) drugs are treated for 6 months. If the four drugs are reduced, RMP and INH will recur after 2 months and treatment will last longer (Zang and Yew, 2009).

Irregular drug exposure will cause spontaneous resistance because *Mycobacterium tuberculosis* has a wall layer that is resistant to acids. Morphologically, the *Mycobacterium tuberculosis* exposed to the anti-TB drug INH will experience changes in size and shape (Widodo, 2016).

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Observed Treatment Short-course (DOTS)

2. Method

This research was carried out in the Microbiology Laboratory of Campus III, Semarang Ministry of Health Polytechnic, Semarang Community Health Center and Health Laboratory and Testing Center of the Central Java Medical Center. This study used a non-experimental cross-sectional design with no descriptive analytic intervention that takes phlegm specimens from patients suffering from tuberculosis. The number of samples was 9 specimens with inclusion criteria not to be converted for 2 months, microscopic examination, culture test, and Mycobacterium tuberculosis drug sensitivity test (DST).

3. Result and Discussion

Sputum samples obtained from Semarang Community Health Center (BALKESMAS Semarang) were carried out with microscopic examination directly by making sediaan preparations measuring 3 x 2 cm and then continued with Ziehl Neelsen staining and finally they were cultured on Jensen lowenstein media for 6-8 weeks at 37°C.

Table 1. Results of Ziehl Neelsen Stain and Culture

No	Sample code	ZN Stain	Culture results	PNB	Niacin	Catalase heat	DST INH test
1	X01	N	NGR	-	-	-	-
2	X02	N	NGR	-	-	-	-
3	X03	N	NGR	-	-	-	-
4	X04	N	NGR	-	-	-	-
5	X05	N	1 C	N	P	N	S
6	X06	N	NGR	-	-	-	-
7	X07	N	2 C	N	P	N	S
8	X08	1+	5 C	N	P	N	S
9	X09	N	NGR	-	-	-	-

N : Negative , P : Positive , C : Colony, NGR: No Growth

The results in the table show that during intensive treatment and insertion, if the patient did not convert ZN's direct sputum examination to positive results, there is a possibility that there were still surviving *M. tuberculosis* in the sputum of the patients undergoing treatment

therapy. The Percentage of culture results from nine patients was 33.3% of the patients who had sputum with *M. tuberculosis* which can grow in LJ media.

a. Size of *M. tuberculosis*

The morphological variations of *M. tuberculosis* are classified into two categories that are often encountered in the exponential phase with the growth of stems with variations in form V, Y or branching. When under stress conditions or unfavorable environments, they are round, oval, or crooked to resemble the letter L (Velayati and Farnia, 2012; Daniel, 2009).

Table 2. size and morphology damage

Sample	Growth Colony	Average Size	Normal Percentage (%)	Abnormal Percentage (%)
H37Rv	300	2.08	100 %	0
X05	1	1.82	0	100 %
X07	2	1.87	0	100 %
X08	5	1.85	0	100 %

The results of the table show that the percentage of growth rates indicates that *M. tuberculosis* from pure iculture had more growth compared to samples from sputum of TB patients who did not convert treatment. The size decreased due to exposure to the drug so that the size of *M. tuberculosis* underwent this shortening because metabolic rates in response to exposure (stress) tended to be favorable for continuous growth under conditions of nutrient deficiencies or other factors that could cause imbalances in cellular metabolism (Baek et al, 2011).

The size of *M. Tuberculosis* shrinks when it is compared with pure culture. When experiencing treatment, the average size is 1.82 - 1.87 µm while the pure culture is 2.08 µm.

b. Morphology of *M. tuberculosis* with ZN staining

Observations using digital cameras are used to improve the image by adjusting the brightness, contrast and color of the image so that the pixel value is good (Mukti and Kale, 2011).

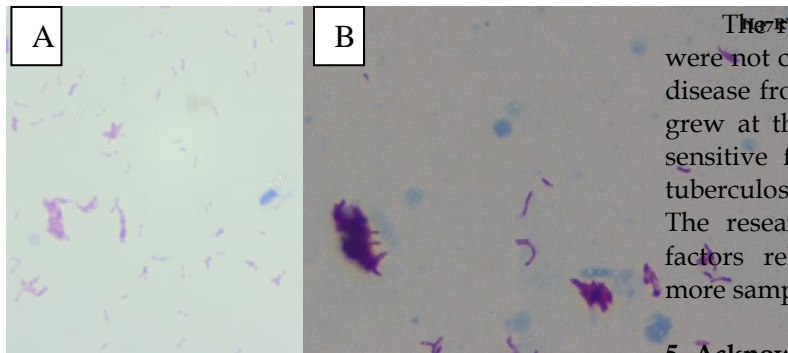


Figure 1. Morphology of *M. tuberculosis* with ZN staining **A.** treatment without conversion, **B** .culture wheel tap H37Rv

Figure A shows *M. tuberculosis* of patients who had undergone treatment without conversion showing that the bacteria was slightly tingling, slightly oval

Figure B shows *M. tuberculosis* from pure culture which had not been exposed to long rod bacterial OAT.

c. DST test (drug susceptibility test) *M. tuberculosis*

Examination of culture with the drug susceptibility test from 9 cultures carried out all the results of sensitive shows can be seen from the table below.

Table 3. DST test (drug susceptibility test) *M. tuberculosis*

Sampel	Prosentase Resistensi			
	INH	Rif	Enta	Strep
X01	-	-	-	-
X02	-	-	-	-
X03	-	-	-	-
X04	-	-	-	-
X05	S	S	S	S
X06	-	-	-	-
X07	S	S	S	S
X08	S	S	S	S
X09	-	-	-	-

- =indicates no growth
S = indicates sensitive drug

The results of the examination in the table show that all samples grown from culture results were still sensitive to first-line OAT with concentrations of streptomycin 4 mg/l, INH 0.2 mg/l, Rifampicin 40 mg/l, Ethambutol 2 mg/l, so that the first line drug can still be used for treatment.

4. Conclusion and Sugestion

The results of examination of patients who were not converted during OAT treatment of TB disease from 9 samples indicated that 3 samples grew at the time of culture; and DST test was sensitive for all morphological changes of *M. tuberculosis* during the treatment process. The research was carried out by considering factors related to medication adherence and more sample coverage.

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