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Mycobacterium tuberculosis resistance pattern against first-line drugs in patients from urban area

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ABSTRACT

Objective/Background: Tuberculosis (TB) infection is still a major public health burden in Indonesia. TB cases in Indonesia constitute 35% of all the TB cases detected worldwide and the prevalence of TB drug resistance in this country is approximately 3%. The aim of this study was to evaluate the resistance of *Mycobacterium tuberculosis* to first-line TB drugs among isolates from clinical specimens from a hospital in an urban area.

Methods: This laboratory-based study was conducted in Tangerang District, Indonesia, from January 2011 to December 2014. Sputum and other clinical specimens were obtained from patients with pulmonary and extrapulmonary TB. The specimens were stained with Ziehl–Neelsen, inoculated on Löwenstein–Jensen media for 6–8 weeks, and tested for sensitivity against first-line TB drugs [isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and streptomycin (SM)].

Results: All TB patients in this study lived in urban areas with male preponderance. Of the 127 *M. tuberculosis* isolates collected, 22% showed resistance to first-line TB drugs. Among these resistant isolates, 20.5% showed resistance to at least one of the first-line TB drugs and 0.8% showed multidrug resistance (MDR). Resistance to EMB, INH, RIF, and SM was seen in 6.3% 6.3%, 4.7%, and 1.6% of isolates, respectively. Polyresistance to EMB and INH, EMB and RIF, and EMB, INH, and RIF was seen in 0.8% of the isolates, respectively.

Conclusion: Our study confirms that drug resistance, including MDR, observed against all first-line TB drugs was a real threat in the management of TB infection in Indonesia. The resistance pattern identified in this study could assist clinicians in providing appropriate treatment regimen to TB patients and improve their clinical outcome.

Introduction

Tuberculosis (TB) is a disease caused by acid-fast bacilli *Mycobacterium tuberculosis*, which infects the lungs (pulmonary TB) and other organs (extrapulmonary) such as the brain, spine, lymph nodes, abdomen, genitourinary system, skin, and joints [1]. Drug-resistant-TB (DR-TB), especially multidrug-resistant TB (MDR-TB) and extensive

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drug-resistant-TB, has become a major public health problem in many countries. According to the 2014 World Health Organization Global Report, of the estimated 9 million TB cases in the world, approximately 56% were from South-East Asia and Western Pacific regions. Of these TB cases, 3.5% of new cases and 20.5% of retreatment cases were attributed to MDR-TB [2]. Indonesia is one of the countries with the highest TB prevalence, with 430,000 new TB cases reported every year, and an estimated MDR-TB proportion of 1.9% among new cases and 12% among retreatment cases [2]. The directly observed treatment, short course (DOTS) strategy implemented since 1995 by the Indonesian National TB Program (NTP) has enabled Indonesia to reach the global target for new smear-positive TB case notification rate—73 cases/100,000 population in 2010 [3]. Routine monitoring of DR-TB is very important because of the limited availability of first-line TB drugs, the high price of second-line TB drugs, and lack of facilities in most health-care centers in Indonesia that could detect DR-TB or have access to second-line TB treatment [4]. Therefore, data on local TB drugs resistance are essential for patient management and selection of appropriate regimen for patients suspected to have the resistance strain, as this helps to prevent the spread of resistant TB [2,5]. Thus, the aim of this study was to evaluate the resistance of *M. tuberculosis* to first-line TB drugs among isolates from clinical specimens from a hospital in an urban area of Indonesia (Tangerang District).

Materials and methods

This study was conducted in Siloam General Hospital, Tangerang District, Indonesia. Tangerang is a city in Banten Province, located approximately 25 km from Jakarta. The study area is one of the largest cities in Indonesia with a population of approximately 2 million distributed over an area of 164.55 km^2. The Disease Control, Prevention and Health Environment Bureau reported that in 2012, Banten Province had 286.7 new TB cases/100,000 population, and the crude death rate in this region was 1.1% [6]. Patients in the participating hospital represented a wide range of socioeconomic strata.

Epidemiological data of TB patients and microbiological data of *M. tuberculosis* from various clinical specimens and its resistance to first-line TB drugs were retrospectively collected from January 2011 to December 2014. Sputum was collected early morning on 3 consecutive days. Other specimens were collected from bronchial fluid, cerebrospinal fluid, urine, and pus from abscess. The diagnosis of pulmonary and extrapulmonary TB was made by clinicians based on history, physical examination, chest radiography, and positive acid-fast bacilli smear, and/or culture [7,8].

Drug-susceptibility testing

All specimens underwent decontamination and homogenization using sodium hydroxide at a final concentration of 2% followed by Ziehl–Neelsen staining [9,10]. The drug-susceptibility testing against first-line TB drugs was carried out using the proportional method after inoculating the specimens on Löwenstein–Jensen (LJ) medium and incubating them at 37 °C. The LJ medium was observed two times a week, for 6–8 weeks, to check for growth of the pathogen. The growth was compared with the international standard strain of *M. tuberculosis* H37Rv, which is used to detect resistance [10,11]. The drug susceptibility was determined as the percentage of colonies that grew on the following concentration of four first-line TB drugs: isoniazid (INH), 0.1 μg/mL, 1 μg/mL, and 10 μg/mL; rifampicin (RIF), 2.5 μg/mL, 5 μg/mL, and 20 μg/mL; ethambutol (EMB), 1 μg/mL, 10 μg/mL, and 100 μg/mL; and streptomycin (SM), 1 μg/mL, 10 μg/mL, 100 μg/mL, respectively [10].

Definition

Any drug resistance was defined as resistance to one or more first-line TB drugs (INH, RIF, EMB, and SM). Monoresistance was defined as resistance to only one first-line TB drug, and polyresistance was defined as resistance to at least two or more first-line TB drugs except the INH and RIF combination [8,12]. MDR-TB was defined as resistance to the two key first-line TB drugs, namely, INH and RIF [2,5].

Results

The total number of positive culture for *M. tuberculosis* was 127 (15, 37, 45, and 30 cultures collected consecutively in 2011, 2012, 2013, and 2014, respectively). All patients in this study lived in urban areas (55.1% male and 44.9% female patients). Other profiles of TB patients are presented in Table 1.

The proportion of resistance to first-line TB drugs during 4-year observation period is presented in Table 2. Most of the *M. tuberculosis* isolates were collected from sputum specimens (87.4%). Isolates collected from the cerebrospinal fluid of patients with brain infection caused by *M. tuberculosis* constituted 2.4% of the total specimen. The highest prevalence of any drug resistance was noted in 2012 and 2014. Twenty eight (22%) of the 127 *M. tuberculosis* isolates collected showed resistance; among these, 24 (18.9%) were monoresistant and two (1.6%) were polyresistant. There was only one isolate of MDR-TB, which was found in 2014. The overall resistance to single drugs was noted in eight isolates (6.8%; against EMB and INH); six (94.7%) isolates were resistant to RIF and two isolates (1.6%) were resistant to SM. Polyresistance to EMB and INH and EMB and RIF was noted in 0.8% of the isolates, respectively ($n = 1$ each).

Discussion

In last decades, Indonesia has been identified as one of the countries with the highest TB prevalence in the world. The incidence of TB has decreased as reported by the NTP report. However, the emergence and spread of DR-TB threatens TB control and has become a major public health problem in Indonesia. Development of DR-TB, including MDR, is attributed to poor patients' compliance, inappropriate TB drug regimen, inadequate laboratory facilities for drug-susceptibility testing, and acceleration of human immunodeficiency virus epidemic [3,5,13,14].

In this study, 18.9% of *M. tuberculosis* isolates were resistant to at least one first-line TB drug. This number was lower than

Table 1 – Epidemiological and clinical profile of tuberculosis patients.

	Year				Total cases
	2011	2012	2013	2014	$n = 127$, n (%)
Female	7	12	21	17	57 (44.9)
Male	8	25	24	13	70 (55.1)
Type of specimen					
Bronchial fluid	2	2	1	3	8 (6.3)
Sputum	11	33	42	25	111 (87.4)
Cerebrospinal fluid	1	1	1	0	3 (2.4)
Pus	1	0	1	2	4 (3.1)
Urine	0	1	0	0	1 (0.8)

Table 2 – Resistance pattern of *Mycobacterium tuberculosis* to first-line TB drugs from 2011 to 2014.

TB drug-resistance pattern[a]	Year				Total cases
	2011 $n = 15$, n (%)	2012 $n = 37$, n (%)	2013 $n = 45$, n (%)	2014 $n = 30$, n (%)	$n = 127$, n (%)
Susceptibility	14 (93.3)	27 (73.0)	35 (77.8)	23 (76.7)	106 (83.5)
Any drug resistance[b]	1 (6.7)	10 (27.0)	10 (22.2)	7 (23.3)	28 (22.0)
INH	1 (6.7)	3 (8.1)	3 (6.7)	2 (6.7)	9 (7.1)
RIF	0 (0.0)	2 (5.4)	2 (4.4)	3 (10.0)	7 (5.5)
EMB	0 (0.0)	4 (10.8)	4 (8.9)	2 (6.7)	10 (7.9)
SM	0 (0.0)	1 (2.7)	1 (2.2)	0 (0.0)	2 (1.6)
Monoresistance[c]	1 (6.7)	10 (27.0)	6 (13.3)	7 (23.3)	24 (18.9)
INH	1 (6.7)	3 (8.1)	2 (4.4)	2 (6.7)	8 (6.3)
RIF	0 (0.0)	2 (5.4)	1 (2.2)	3 (10.0)	6 (4.7)
EMB	0 (0.0)	4 (10.8)	2 (4.4)	2 (6.7)	8 (6.3)
SM	0 (0.0)	1 (2.7)	1 (2.2)	0 (0.0)	2 (1.6)
Polyresistance[d]	0 (0.0)	0 (0.0)	2 (4.4)	0 (0.0)	2 (1.6)
RIF + EMB	0 (0.0)	0 (0.0)	1 (2.2)	0 (0.0)	1 (0.8)
INH + EMB	0 (0.0)	0 (0.0)	1 (2.2)	0 (0.0)	1 (0.8)
MDR[e]	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)	1 (0.8)

EMB = ethambutol; INH = isoniazid; MDR = multidrug resistance; RIF = rifampicin; SM = streptomycin.
[a] Local data in a hospital.
[b] Any drug resistance: resistance to any of the first-line TB drugs.
[c] Monoresistance: resistance to only one first-line TB drug.
[d] Polyresistance: resistance to at least two or more first-line TB drugs except INH and RIF combination.
[e] MDR: resistance to at least INH and RIF.

that reported in a previous study conducted by Massi et al. [15] in Makassar, Indonesia, where the proportion of *M. tuberculosis* resistant to at least one first-line TB drug reached 40% in new and retreatment cases. The proportion of DR in this study was also lower than that reported Nana et al. [16] in China where 33.3% isolates were resistant to all first-line TB drugs. The resistance to TB drugs in this study was comparable with other studies in Asia where the proportion of resistance ranged from 18.7% to 30.2% [16,17].

The proportion of resistance to INH and EMB was 6.3%, which was similar to a previous study in Iran, where proportion of resistance to INH was 8% and EMB was 5.5% [18]. Most of the previous studies in South-East Asia and Western Pacific regions showed that the resistance level to INH was higher with a range of 10.2–37% [18,19,20]. Resistance to INH in this study might be due to poor management or the INH resistance prior to the current treatment [17,18]. Previous studies conducted by Ayaz et al. [7] and Sasmono et al. [21] reported that the proportion of INH resistance was not associated with ethnicity, but only with socioeconomic strata or limited health-care facilities. The proportion of INH resistance exceeding 10% predicts the development of MDR-TB [17,22]. Therefore, *M. tuberculosis* culture with drug-susceptibility testing for at least INH and RIF should be performed for all TB patients before the start of treatment [2,20]. A meta-analysis by van der Werf and Langendam [23] reported that patients who took inappropriate TB drugs regimen and patients who had treatment failure were on a 27-fold risk of getting MDR-TB. The low proportion of MDR-TB in 2014 in this study was similar to the result from other studies [7,17,20]. This result indicates that the Stop TB program with DOTS and the use of local-specific drug-resistance data are effective in preventing the spread of MDR-TB.

Our study population mainly included patients attending a private general hospital; in addition, our sample size was small, and therefore, this may not be a representative of all

TB patients. Thus, drug-resistance pattern with a larger population is needed to determine the level of MDR, design a standard empirical MDR regimen, and monitor the spread of MDR-TB. The recommendation of appropriate treatment regimen will also improve successful treatment and survival [24].

Conclusion

Our study confirms that DR, including MDR, observed against all first-line TB drugs was a real threat in the management of TB infection. The resistance pattern in this study could assist the clinicians in providing appropriate treatment regimen to TB patients and improve their clinical outcome.

Conflict of interest

The authors declare no potential conflict of interest.

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