Implementation of 25-well culture plates for *M. tuberculosis* drug susceptibility testing in Indonesia

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Abstrak

Saat ini tidak ada metode standar untuk uji kepekaan obat terhadap Mycobacterium tuberculosis di antara laboratorium-laboratorium di Indonesia. Sejak bulan Januari 2002 sampai dengan Januari 2004, kami mencoba menerapkan metode plat dengan 25 sumur yang berisi media middlebrook's (metode Drug Susceptibility Culture Plate (DSCP)) yang digunakan oleh Dutch Supranational Reference Laboratory at the Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands. Pengalaman kami memperlihatkan bahwa metode ini berpotensi memberikan hasil yang lebih baik karena sangat mudah distandardisasi, hasilnya lebih cepat dan dapat memperlihatkan nilai KHM (Konsentrasi Hambat Minimum) yang lebih terperinci. Data dari 364 isolat yang telah diuji dengan metode DSCP memperlihat resistensi terhadap INH, rifampisin, ethambutol, dan streptomisin secara berurutan adalah 21,4%; 19,8%; 15,7%; and 16,5%. Resistensi ganda didapatkan pada 13,2% isolat. ***(Med J Indones 2005; 14: 142-6)***

Abstract

At present, there is no standardized method for Mycobacterium tuberculosis drug susceptibility testing (DST) among laboratories in Indonesia. Since January 2001 to January 2004 we have tried to establish the method of 25-well culture plates with middlebrook's media (Drug Susceptibility Culture Plate (DSCP) method) used by the Dutch Supranational Reference Laboratory at the Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands. Our experience showed that this method potentially gives better result as it can be very well standardized, faster and provides detailed MIC (Minimal Inhibitory Concentration) values. Data from 364 isolates that have been tested by DSCP method showed that resistance to INH, rifampicin, ethambutol, and streptomycin were 21.4%, 19.8%, 15.7%, and 16.5% respectively. Multidrug resistance were found in 13.2% isolates. ***(Med J Indones 2005; 14: 142-6)***

Keywords: *M. tuberculosis, DST, DSCP method*

Drug resistance of *Mycobacterium tuberculosis* (MTB) strains highly contributes to the spread of TB in some countries.[1,2,3,4,5] Little is known about drug resistance of tubercle bacilli in Indonesia, which has not yet been included in the WHO Global Surveillance and Monitoring Project of Drug Resistance.[6]

Poor case-management is probably the most important contributing factor to the development of drug resistance of MTB in Indonesia. Standard procedures for diagnosis and treatment are often not followed.[7]

Logistical problem, time and space hamper large-scale susceptibility testing with conventional methods in many laboratories in Indonesia. Implementation of reliable method for susceptibility testing may provide the means for surveillance of drug resistance, and identification of individual patients with multidrug resistance.[4,7,8]

METHODS

In this study are established a simple method with a semi-automated device to produce small 25 chamber culture plates filled with transparent medium (7H10 Middlebrook's) and different dilutions of several antituberculous drugs (Drug Susceptibility Culture Plate/ DSCP method).

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Preparation of the plates: The precise dilutions of 6 antituberculous drug (isoniazid, rifampicin, amikacin, para-amino-salicylic acid, ethambutol, streptomycin) are prepared and added to the middlebrook's media.[9] The solution are distributed to the 25 chambers using semi automated device (Figure 1). For control, 2 wells are filled with media without antituberculous drug. The obtained plates contain a series of final concentration of the drug is shown in Figure 2.

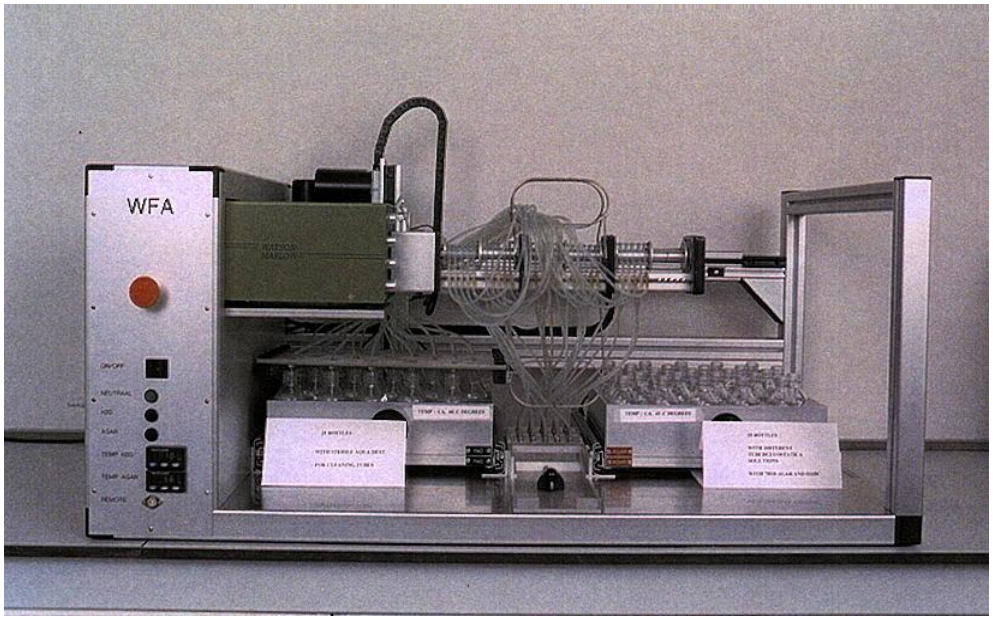

Figure 1. The semi automated device to prepare drug susceptibility culture plate

Contr	1/100 Contr	**AMK 5**	INH 0.1	**INH 0.2**
INH 0.5	INH 1	INH 2	RIF 0.1	RIF 0.2
RIF 0.5	**RIF 1**	RIF 2	RIF 5	**PAS 1**
ETB 1	ETB 2	**ETB 5**	ETB 10	ETB 20
STR 1	STR 2	**STR 5**	STR 10	STR 20

Figure 2. Mirrowed 25 chamber plate after incubation and concentration each of 25 chamber plates in mg/l (INH= isoniazid, RIF= rifampicin, AMK= amikacin, PAS= para-amino-salicylic acid, ETB= ethambutol, STR= streptomycin) (the numbers written in bold are the minimal inhibitory concentration (MIC) of each drug)

Inoculation of the plates: A bacterial suspension of McFarland 1 (2-10 x 10^5 cfu) is made by suspending *M. tuberculosis* in a bottle containing water and glass beads. The beats will help suspending the bacteria homogeneously under shaking conditions. This suspension is vigorously shaken for 20 minutes and 10 µl is brought on 24 chambers, using a repetitive pipette (stepper). One of the control chambers is not inoculated with the McFarland 1 suspension, but inoculated with a 1/100 dilution of this suspension (1% control).[10,11,12] The plates are covered by a lid placed in a perpendicular position and incubated overnight to evaporate exceeding liquid, by leaving a small space in between the lid and the plate itself. After one night the lid is closed and the plates are incubated at 37^0 C.

Reading and interpretation of the results: Reading is only performed when the controls show growth. The growth is always compared with the two control chambers. Five days after inoculation, preliminary resistance profile can sometimes already be preliminary determined, especially for atypical Mycobacteria (rapid growers). The definitive MIC values are read at day 12^{th}. The last reading at 19^{th} day is only for control whether there is contamination, or for slow growing Mycobacteria. The MIC value is the value on which the growth is reduced until almost zero, with at least 99% reduction (less than the 1/100 control). The values are converted into results; sensitive or resistant.

Quality control of the plates[7]: Quality control on every batch of plates preparation include internal, batch, and second-line control.

Internal control: in every plate the growth of inoculum is controlled on two control chambers without drug. This represents the internal growth control of the plate; all growth on every chamber in the plate is compared with the two control chambers. Growth in the chambers show whether the strain grows sufficiently to give results (control chamber) and what the cut off amount of bacteria is (1/100 control chamber).

Batch control: in every batch of plates four control strains with a known susceptibility pattern are inoculated. The MIC's of the control strains give results on the critical value, when the concentration of antituberculous is right.

Second-line control: Isolates were exchanged with the RIVM laboratories and the results are evaluated or some isolates were tested on 2 plates made in FKUI and made in RIVM.

RESULTS

There are tested 364 samples have been collected from January 2002 to January 2004 in Department of Microbiology, Medical Faculty, University of Indonesia, Jakarta; BLK, Bandung; and Tangerang. Data from 364 isolates that had been tested by DSCP method showed resistance to INH, rifampicin, ethambutol, and streptomycin respectively 21.4%, 19.8%, 15.7%, and 16.5%. Multidrug resistance were found in 13.2% isolates (Table 1, Figure 3).

For second-line control (Table 2): 31 isolates were inoculated in both plates prepared in Jakarta and in RIVM Netherlands was done. The results showed agreements between 93.5% (ethambutol) to 100% (INH and rifampicin).

CONCLUSION

Twenty-five well culture plates method for *M. tuberculosis* drug susceptibility testing was reliable method to provide the information of *M. tuberculosis* drug resistance surveillance and therapy guidance in Indonesia, where TB is highly prevalent. Implementation of this method in Indonesia has been proven successfully.

Since January 2003, 25-well culture plates have been produced routinely in Department of Microbiology Medical Faculty University of Indonesia in Jakarta, and used Dept Microbiology FKUI Jakarta, BLK Bandung, and Tangerang. From 364 isolates that had been tested by 25-well culture plates method showed resistance to INH, rifampicin, ethambutol, and streptomycin respectively 21.4%, 19.8%, 15.7%, and 16.5%. Multidrug resistance were found in 13.2 % isolates.

Reports from other investigators showed that prevalence of resistance cases are increased from year to year in the world. This data suggest that drug resistance is a problem in tuberculosis treatment, and continuous monitoring of drug resistance must be done.

Table 1. Susceptibility pattern by DSCP method to Oral Anti Tuberculous (OAT) drugs in Indonesia (Jakarta, Bandung, Tangerang)

OAT	Sensitive (S)	Intermediate (I)	Resistant (R)	Contaminated	S+I /Total	R/Total
INH	263	23	78	0	286/364	78/364 (21.4%)
RIF	288	4	72	0	292/364	72/364 (19.8%)
ETB	263	43	57	1	306/363	57/363 (15.7%)
STR	284	19	60	1	303/363	60/363 (16.5%)
Multidrug Resistance Tuberculosis (MDR-TB) = 48/ 364 (13.2%)						

Note: (INH= isoniazid, RIF= rifampicin, ETB= ethambutol, STR= streptomycin)

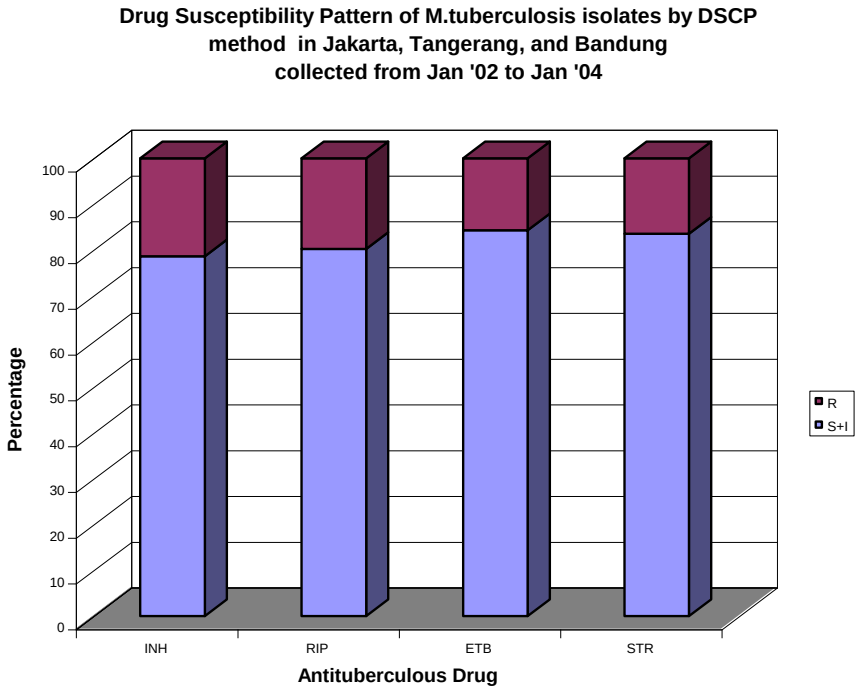

Figure 3. Drug susceptibility pattern of M. tuberculosis by DSCP method in Indonesia (Jakarta, Bandung, Tangerang)

Table 2. Agreement of drug sensitivity pattern tested in RIVM and Jakarta plates

Susceptibility to drug	Agreement
INH	100%
RIF	100%
ETB	93.5%
Strep	96.8%

Table 3. Percentage of *M. tuberculosis* resistance to each drug in Microbiology Department FKUI (by conventional method)

No.	Results	2000	2001	2002	2003
1.	Resistant (I)	1 (0,7%)	1 (0,6%)	2 (0,8%)	5 (2,4%)
2.	Resistant (R)	4 (2,9%)	16 (9,4%)	24 (10,0%)	7 (3,3%)
3.	Resistant (E)	0 (0%)	0 (0%)	1 (0,4%)	3 (1,4%)
4.	Resistant (S)	0 (0%)	1 (0,6%)	30 (12,4%)	31 (14,8%)
	Amount of isolates (= n)	136/1714	170/1823	241/2103	209/2170
	MDR-TB	0 (0%)	1 (0,6%)	8 (3,3%)	12 (5,7%)

Note: (INH= isoniazid, RIF= rifampicin, ETB= ethambutol, STR= streptomycin)
From Rosana Y et al (2005)

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