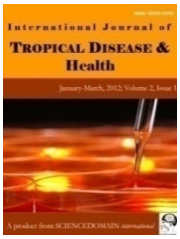

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MODS Culture for Primary Diagnosis of Tuberculous Meningitis and HIV-Associated Pulmonary Tuberculosis in Indonesia

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Authors' contributions

This work was carried out in collaboration between all authors. Author LC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author JA managed the specimens and data collection in laboratory. Authors SD and ARG managed the inclusion of TB meningitis patients in the hospital as well as the patient information. Author DAJM reviewed the manuscript before submission. Authors SM and TK performed the study in the laboratory. Author IP coordinated the laboratory work. Author ML managed the inclusion of HIV-infected pulmonary TB in the hospital as well as the patient information. Authors BA and RvC designed the study and reviewed the data analysis and the manuscript. All authors read and approved the final manuscript.

Short Communication

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ABSTRACT

Aims: To compare the microscopic observation drug susceptibility (MODS) culture with microscopy and solid culture for diagnosis of TB meningitis and HIV-associated pulmonary tuberculosis (TB).
Study Design: Comparative study.
Place and Duration of Study: Department of Clinical Pathology, Hasan Sadikin Hospital, Bandung, Indonesia, between 2010 and 2012.
Methodology: Two groups of patients were included. The first group consisted of 167 consecutive HIV-infected patients presenting with suspected pulmonary TB. The second group consisted of 88 patients with clinical suspicion of TB meningitis. Sputum samples from HIV-associated pulmonary TB patients and cerebrospinal fluid (CSF) from patients with TB meningitis were analyzed using microscopy of Ziehl-Neelsen (ZN) stained smears, culture on solid medium (Ogawa), and MODS culture.
Results: MODS showed the highest detection rate in both patient groups. Among HIV-associated pulmonary TB patients, positivity of MODS was 31.2% compared with 26.9% for Ogawa and 20.6% for ZN. Among TB meningitis patients, positivity of MODS was 41.2% compared with 38.8% for Ogawa and 8.3% for ZN. The median time to culture positivity was significantly shorter for MODS compared to Ogawa, both for sputum (median 11 vs 21 days) and CSF (14 vs 33 days). In 14 days, MODS detected significantly more cases compared with Ogawa in both patients group (79.2% vs 2.4% and 68.6% vs 0%, respectively). Laboratory staff readily used MODS after two weeks of training.
Conclusion: We were able to implement MODS culture as a robust, sensitive, and rapid method for diagnosis of HIV-assoicated pulmonary TB and TB meningitis in a hospital setting in Indonesia. Further studies may be needed to assess the feasibility of MODS culture in other settings and assess its impact on case detection and timely treatment of both forms of TB.

Keywords: MODS culture; primary diagnosis; HIV-associated pulmonary TB; TB meningitis; Indonesia.

1. INTRODUCTION

Diagnosis of tuberculosis (TB) is often difficult, especially among HIV-infected patients [1] and among patients with extrapulmonary TB [2]. Microscopy is insensitive and conventional culture is more sensitive but slow. Commercial liquid culture systems, which can shorten the time to detect growth of *Mycobacterium tuberculosis* require sophisticated instrumentation and are more costly [1]. The World Health Organization has recently endorsed the Xpert MTB/RIF which is a nucleic acid amplification-based test. The Xpert test is a rapid and simple method for TB diagnosis and detection of rifampicin resistance. However, it is less sensitive for diagnosis of paucibacillary disease [2], it is relatively expensive, and cannot evaluate treatment response. In addition, culture is still needed to confirm the results of drug susceptibility testing (DST). Therefore, there is a need for rapid, efficient, and inexpensive culture-based diagnostic tools.

One of several recently developed methods for diagnosis and DST of TB is the microscopic observation drug susceptibility (MODS) culture. MODS uses broth culture and an inverted light microscope to rapidly detect specific growth of *M. tuberculosis*. MODS can be used for

primary diagnosis as well as DST. With regard to primary diagnosis, a few studies have examined MODS for diagnosis of pulmonary TB [1,3,4], and one study has evaluated MODS for diagnosis of TB meningitis [5]. These studies suggest that MODS culture, with its high sensitivity, speed and low price, and its potential to add DST, may be a suitable alternative for diagnosis of paucibacillary forms of TB, and for use in low-resource settings.

However, for successful implementation more studies are needed, in different patient groups and settings. We compared MODS culture with microscopy and solid culture for diagnosis of TB meningitis and HIV-associated pulmonary TB in Indonesia, which has a high burden of TB and a rapidly growing HIV epidemic. Like in other settings, bacteriological confirmation of HIV-associated pulmonary TB and TB meningitis is difficult, and mortality is very high [6,7]. More rapid diagnosis would help timely treatment to improve patient outcome and reduce TB transmission.

2. MATERIALS AND METHODS

This study was conducted between February 2010 and January 2012 at Dr. Hasan Sadikin Hospital, the referral hospital for West Java Province, Indonesia. Written informed consent was obtained from all participants and the study was approved by the ethical committee of the Faculty of Medicine, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia.

We included two groups of patients with suspected TB. The first group consisted of HIV-infected patients, either from the outpatient clinic or the HIV clinic in the hospital, who had symptoms or chest X-ray (CXR) abnormalities suggesting pulmonary TB. From this group one to three consecutive sputum samples were collected per patient prior to initiation of TB treatment. Direct smears were prepared from each sputum specimen for Ziehl-Neelsen (ZN) staining. The remaining sputum specimens were decontaminated by a standard N-acetyl-L-cystein (NaLC)-NaOH method and concentrated by centrifugation at 3000 x g for 15 minutes. For each patient, one sputum culture was made. Sputum specimens from patients who had provided ≥ 1 specimen were mixed before decontamination. The resulting sediments were then resuspended and inoculated into MODS medium as described previously [8,9] and onto two slants of Ogawa solid medium.

The second group consisted of adult patients (≥18 years old) with suspected TB meningitis. A clinical diagnosis of meningitis was based on clinical findings, CSF criteria or both [10]. Five to ten milliliters of CSF was obtained and divided into two tubes. The first tube (0.5–1 mL) was used for CSF cells, protein and glucose. The second tube (4-10 ml) was concentrated by centrifugation at 3000 x g for 15 minutes. The CSF sediment was used to prepare smears for ZN-staining and was inoculated into MODS medium [5] and onto two slants of Ogawa solid medium.

For this study, MODS were not performed on a daily basis to simplify the technical work, reduce the high work load in our laboratory, and to standardize the time for microscopic observation. After preparation of smears, specimens were stored in 4ºC before they were processed for culture. Positive MODS cultures due to cross-contamination seem very unlikely, as previous studies have shown that cross-contamination is extremely rare in MODS [11]. To further reduce the possibility of cross-contamination and to improve biosafety, MODS plates were sealed in ziplock bags after inoculation.

ZN-staining and microscopic reading was done according to standard guidelines. Ogawa cultures, considered positive when mycobacterial growth of > 1 colony forming unit (CFU) was observed were read twice weekly until 8 weeks after incubation. Mycobacterial growth in MODS plates was examined twice weekly under an inverted light microscope at 40x magnification from day 4 until day 40 after inoculation. Positive MODS cultures were identified by cord formation which is characteristic of *M. tuberculosis* growth.

For quality control of microscopy positive (*M. tuberculosis* H37Rv) and negative (sterile distilled water) controls are included daily. Our laboratory also participates and qualifies for the external quality assurance (EQA) program for smear microscopy provided by the WHO. For mycobacterial culture, we monitor the contamination rate on a monthly basis, as a quality control for the decontamination process. For MODS, negative controls (sample-free liquid medium) were included in each plate, and positive controls (H37Rv) for each run. To minimize the risk of cross-contamination, the positive controls were set up in a separate plate, after all plates with patients samples had been sealed.

The positivity rate of microscopy and mycobacterial culture using Ogawa and MODS was expressed as percentage. For HIV-infected individuals suspected of having pulmonary TB, more than one sputum sample was often available. However, analysis in this group was done per patient, not per individual sample. Time to culture positivity was expressed as median days (interquartile range, IQR). Differences between groups were compared using Chi-square test for proportions with $P < .05$ considered statistically significant.

This study was approved by the ethical committee of the Faculty of Medicine, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia. Written informed consent was obtained from each patient.

3. RESULTS

We included 167 HIV-infected patients screened for pulmonary TB. All patients were antiretroviral therapy (ART)-naive at the time they were screened for TB. Most patients came with advanced HIV infection, with a median CD4 cell count of 51/µl (IQR 10-252). As a second group, we included 88 patients with suspected TB meningitis. Most patients presented with lowered consciousness and focal neurological signs, and with typical CSF findings of moderate pleiocytosis, elevated protein and lowered glucose, 9 patients (11%) were HIV-infected.

MODS culture had the highest detection rate among both groups (Fig. 1). In HIV-infected patients with suspected pulmonary TB, MODS culture of 7/167 patients (4.2%) were contaminated and were excluded from the calculation of positivity rate. Of the remaining 160 patients, MODS culture was found positive in 50 patients (31.2%) compared with 43 patients (26.9%) for Ogawa culture and 33 patients (20.6%) for ZN staining and microscopy; the difference between MODS culture and ZN was statistically significant ($P = .02$). None of the patients whose MODS cultures were contaminated had a positive Ogawa culture. Among 127 ZN smear-negative patients, MODS was positive in 20 patients (15.8%) compared with 15 (11.8%) using Ogawa. Among patients with suspected TB meningitis, MODS cultures of 3 patients (3.4%) were contaminated and were excluded from the analysis. Of the remaining 85 patients, MODS culture also gave the highest detection rate; positive in 35 patients (41.2%) compared with 33 patients (38.8%) for Ogawa and 7 patients (8.3%) for ZN. Both MODS and Ogawa culture were significantly more sensitive compared to ZN ($P < .001$). Of patients whose MODS cultures were contaminated, one patient was positive for the Ogawa

culture. Among 77 smear-negative patients, MODS was positive in 30 (39.0%) and Ogawa in 27 (35.1%) patients. Ten patients in both patient groups were MODS-positive/Ogawa-negative, and 3 and 8 patients, respectively, were MODS-negative/Ogawa-positive. This was thought to be due to the low numbers and clumping of mycobacteria which makes that bacilli are not always uniformly distributed in sample aliquots.

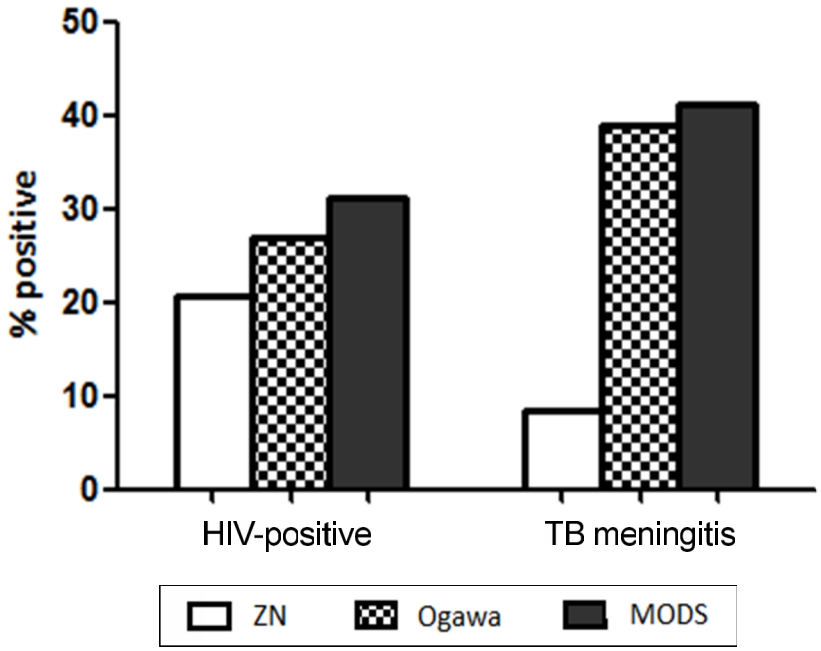

Fig. 1. Positivity rate of microscopy of Ziehl-Neelsen-stained smears, Ogawa solid culture, and MODS culture for suspected pulmonary TB among 160 HIV-infected patients and suspected TB meningitis among 85 patients. Seven patients in HIV-infected pulmonary group and three patients in TB meningitis group were excluded due to contamination in MODS cultures

The median time to culture positivity was shorter for MODS culture than for Ogawa culture in both groups of patients (Fig. 2). Using sputum from HIV-infected patients, positive results for MODS were obtained after a median 11 days (IQR 7-14 days) compared with 21 days (IQR 18-28 days; $P < .001$) for Ogawa. Within 14 days, MODS detected 79.2% of positive samples, compared to only 2.4% for Ogawa ($P < .001$). Among patients with suspected TB meningitis, the median time to culture positivity of CSF samples was 14 days (IQR 11-18 days) for MODS, compared to 33 days (IQR 23-39 days, $P < .001$) for Ogawa. In this group, MODS detected 68.6% of positive cultures within 14 days, while all Ogawa cultures were still negative by this time.

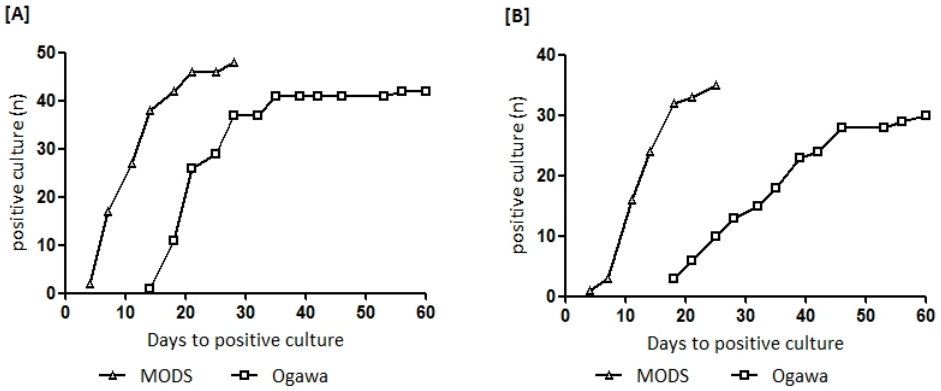

Fig. 2. Positive culture results (n) in time for MODS (∆) and Ogawa (□) among patients with suspected HIV-associated pulmonary TB [A] and suspected TB meningitis [B]

4. DISCUSSION

Bacteriological confirmation of certain paucibacillary forms of TB, like TB meningitis, is problematic. We examined the performance of MODS as a rapid culture-based method for diagnosis of TB meningitis and HIV-associated pulmonary TB in a hospital setting in Indonesia. As comparative tests we used solid culture and microscopy of Ziehl-Neelsen stained smears, the two most widely used methods for TB diagnosis in low-resource settings. For both study groups, MODS culture had the highest positivity rate. In addition, MODS culture was much quicker compared with Ogawa solid culture.

The high positivity rate of MODS culture, which is due to the use of liquid medium, was in line with previous studies [8,12,13]. One previous study reported that MODS was more sensitive than automatic mycobacterial culture for diagnosis of pulmonary TB [3], but this was not confirmed in another study [1]. To our knowledge, only one study evaluated MODS for suspected TB meningitis [5]. This study from Vietnam showed that MODS was a sensitive and simple culture-based method for diagnosis of TB meningitis. Mycobacterial Growth Indicator Tube (MGIT, Becton Dickinson) showed a slightly higher sensitivity in the Vietnam study, but the difference was not significant. A smaller volume of deposit was inoculated into the MODS culture than the MGIT culture, possibly contributing to the slight difference in sensitivity.

In our study MODS culture significantly reduced the time to both positive and negative results. After two weeks, 79.2% of HIV-associated pulmonary TB had been diagnosed with MODS, and 68.6% of TB meningitis, compared with 2.4% and 0%, respectively, using Ogawa solid culture. These findings are in line with previous studies in HIV-associated pulmonary TB [1,3] and TB meningitis patients [5]. With the high sensitivity and rapidity, MODS culture is suitable for the TB screening of HIV-infected patients, as shown by a study in Peru [4].

Among HIV-infected patients examined for pulmonary TB, the positivity rate of ZN was relatively high compared to culture. This is probably caused by the fact that ZN microscopy was performed on all available sputum samples for each patient, while only single Ogawa and MODS culture were performed for every patient.

The contamination rate of MODS culture was around 3-4%. A contamination rate of 2%-3% is acceptable for fresh specimens, but it may be as high as 5%-10% for stored samples [14].

MODS culture is a simple test. Sample preparation for MODS is similar to that required for other culture methods. The microscopic detection of mycobacteria by MODS is technically similar to that of a smear. Our laboratory technicians were able to perform the MODS assay and accurately read the results after two weeks of training. The cord formation, which is characteristic of *M. tuberculosis* in liquid culture, is easy to recognize using inverted microscopy. Certain species of non-tuberculous mycobacteria (NTM), for example *M. chelonae*, may also form cording in liquid culture but this can be recognized by rapid overgrowth by day 5 [3]. Of note, genotyping of > 800 cultures isolated in our laboratory has only revealed a very low number of NTM (<3%) [15].

A possible limitation of this study is that we did not include automated liquid culture (MGIT), which is recommended as diagnostic standard for culture, as the comparative test. The few studies comparing MODS culture with MGIT showed slightly different results [1,3,5]. Furthermore, our study was performed in a single laboratory. However, as this is a busy hospital laboratory of a large hospital, our results suggest that MODS can be implemented in high-burden settings. Finally, we did not evaluate MODS for DST. Therefore, further studies may be needed to assess the feasibility of MODS culture in other settings, and assess its impact on case detection and timely treatment of paucibacillary forms of TB like TB meningitis.

5. CONCLUSION

MODS culture showed a high sensitivity and rapidity (compared with solid culture), simplicity, and potential low cost (compared with automated liquid systems) for diagnosis of HIV-associated pulmonary TB and TB meningitis in a hospital setting in Indonesia. As such, this seems a reliable method to help implement *M. tuberculosis* culture more widely and speed up TB diagnosis in TB endemic settings.

CONSENT

All authors declare that written informed consent was obtained from the patients.

ETHICAL APPROVAL

This study was approved by the ethical committee of the Faculty of Medicine, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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