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Eleven-year experience on anti-TB drugs direct susceptibility testing from Siriraj Hospital, Thailand

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ABSTRACT

The early detection of drug-resistant tuberculosis (TB) strains is of utmost importance for patient management and effective TB control programs. This study aimed to demonstrate the performance of direct drug susceptibility testing (DST) performed in our laboratory in the past 11 years. The direct DST was performed on Middlebrook 7H10 medium using isoniazid (INH) and rifampicin (RIF), and the results were compared with those obtained from indirect DST (gold standard). The direct DST was performed with 15,598 smear-positive sputum samples, of which 11,284 (72%) yielded reportable results. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated and revealed 89%, 99%, 95%, and 99%, respectively, for RIF and 90%, 98%, 93%, and 97%, respectively, for INH. Direct DST results could be reported within 1 month after sample processing. This method was also shown to be suitable for use in resource-limited countries, particularly in settings with high numbers of TB cases.

1. Introduction

An increasing incidence of drug-resistant tuberculosis, particularly multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB), makes the control of tuberculosis (TB) more difficult. The World Health Organization (WHO) estimates that, in 2008, there were 440,000 MDR-TB cases and 150,000 deaths. XDR-TB was found in 5.4% of MDR-TB cases (WHO, 2010). MDR-TB is defined as a strain that is resistant at least to rifampicin (RIF) and isoniazid (INH), whereas XDR-TB is an MDR-TB strain that additionally resists any fluoroquinolone and an injectable drug (e.g., amikacin, kanamycin, or capreomycin). The early detection of drug-resistant strains is of utmost importance for patient management and for effective TB control programs.

Conventional methods for detecting drug-resistant strains depend on the primary isolation of tubercle bacilli from specimens and the subsequent performance of indirect drug susceptibility testing (indirect DST). This takes approximately 6–8 weeks, which is too long a period to allow timely decisions on patient management, especially in cases of MDR-TB. Direct drug susceptibility testing (direct DST) is well established and has recently been performed in liquid media, and it is compatible with automated culture systems. The direct DST method is a rapid and reliable method compared with indirect DST (Kent and Kubica, 1985; Goloubeva et al., 2001; Kim, 2005; NCCLS, 2003; Siddiqi et al., 2012). Direct DST can be simultaneously performed when a processed specimen is inoculated for isolation, and it may reduce the time required to detect drug resistance. The present study demonstrated the performance of direct DST performed in our laboratory for the past 11 years. The direct DST results were compared with those from indirect DST, which was used as a gold standard.

2. Materials and methods

2.1. Sputum samples

A total of 29,508 sputum samples were sent between 2001 and 2011 from 288 hospitals in 46 provinces of Thailand for direct and indirect DST. DST was performed in the Drug-Resistant Tuberculosis Research Laboratory, Drug-Resistant Tuberculosis Research Fund, Siriraj Foundation, Faculty of Medicine Siriraj Hospital, Mahidol University. The workflow for susceptibility testing was illustrated in Fig. 1.

2.2. Sputum processing

All sputum samples were processed following the standard NALC-NaOH method for digestion, decontamination, and concentration

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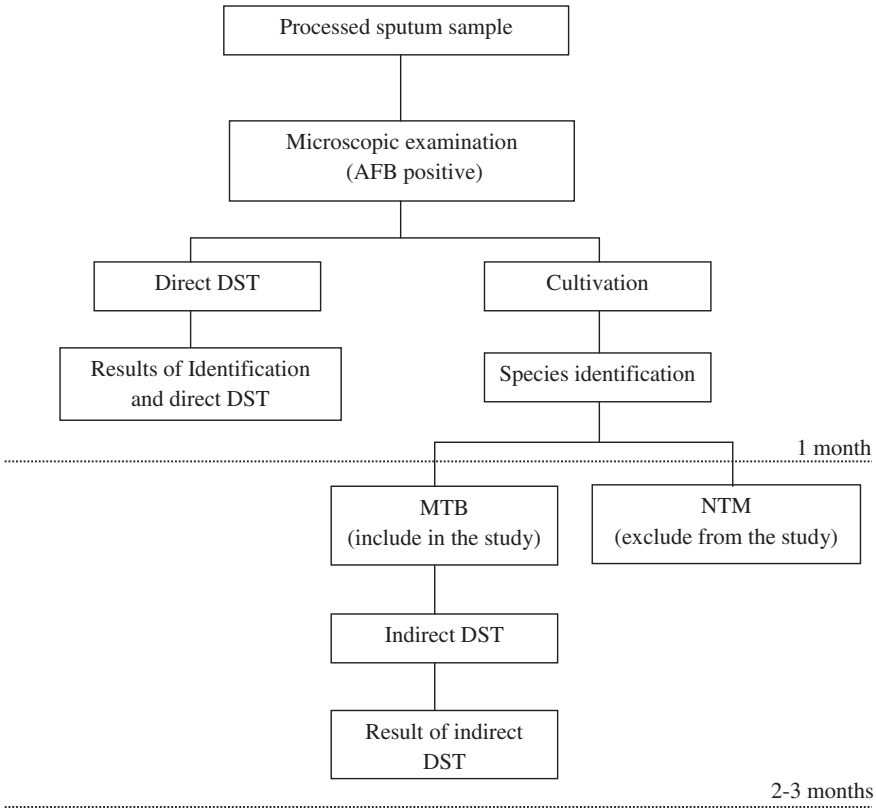

Fig 1. Diagram illustrated the workflow of direct and indirect DST performing in this study.

(Kent and Kubica, 1985). The concentrated sediment was resuspended in 1 mL of 0.85% saline solution and/or phosphate buffer (pH 6.8). A smear was prepared for acid-fast staining. Löwenstein-Jensen (LJ) medium and Middlebrook 7H10 medium (Difco, Detroit, MI, USA) were inoculated according to the standard laboratory procedure for primary isolation and for direct DST, respectively (Kent and Kubica, 1985).

2.3. Acid-fast bacilli (AFB) staining

All sputum samples were stained using the Kinyoun method. Smears were graded according to WHO/International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines (WHO, 1998): as number of cells (1–9 AFB/100 fields), 1+ (10–99 AFB/100 fields), 2+ (1–10 AFB/field), and 3+ (more than 10 AFB/field).

2.4. Primary isolation and identification

LJ medium was used for primary isolation of mycobacteria. Cultures were incubated at 37 °C for a maximum of 8 weeks. Colonies obtained from the cultures were confirmed for the presence of mycobacteria by AFB staining. DNA from AFB-positive colonies was prepared, and mycobacterial species were identified using an in-house multiplex PCR as previously described (Chaiprasert et al., 2006). Only cultures confirmed as *Mycobacterium tuberculosis* or *M. tuberculosis* complex were included in the study. This study was approved by the Siriraj Ethics Committee, Mahidol University, Bangkok, Thailand (Certificate of Approval no. Si 208/2005).

2.5. Drug susceptibility testing

Direct DST was conducted using the disk elution method on Middlebrook 7H10 medium (Difco, Detroit, MI, USA) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) (BBL, Becton Dickinson, USA), as recommended by the CLSI (former NCCLS) (NCCLS, 2003). Briefly, RIF (5 μg) and INH (1 μg) drug discs were dispensed aseptically into the centers of individual quadrants of sterile plastic dishes. Exactly 5.0 mL of sterile, tempered (52 °C), complete M7H-10 medium was pipetted over each disc, and the plates were left overnight at room temperature to permit uniform diffusion of the drug. The inocula were prepared by diluting the processed samples with sterile distilled water (10^{-1} and 10^{-3} for smear 3+, undiluted and 10^{-2} for smear 2+, undiluted for smear 1+); the dilutions were inoculated onto each quadrant and onto a quadrant containing drug-free medium, which was used as a control. The plate was incubated at 37 °C until colonies appeared on the control quadrant (approximately 2–4 weeks). Resistance was reported when the number of colonies on the drug-containing quadrant was ≥1% of the number on the drug-free control quadrant.

Indirect DST was used as a gold standard method in this study and was conducted in the same manner as direct DST, except that the inoculum was prepared by suspending the *M. tuberculosis* cells in Middlebrook 7H9 medium at a turbidity adjusted to match that of the MacFarland No. 1 standard and diluted to 10^{-2} and 10^{-4} in sterile distilled water. These 2 dilutions were inoculated onto each quadrant and onto a quadrant containing drug-free medium.

2.6. Quality control

M. tuberculosis H37Rv strain ATCC 27294 was used as the control for both direct and indirect DST. This strain was used each time a batch of DST was initiated. If any resistance was observed in the control strain, none of the results in that batch were interpreted. All results were read and interpreted by experienced and well-trained laboratory technicians and verified by the second reader before reporting.