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ORIGINAL ARTICLE

Slide drug susceptibility test for the detection of multidrug-resistant tuberculosis in Bangladesh

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Abstract The present study attempted to comparatively assess and establish a suitable detection method of multi-drug-resistant tuberculosis (MDR-TB) from previously treated TB cases in Bangladesh. Of 130 Zeihl–Neelsen smear-positive fresh sputum specimens, 112 samples were found to contain viable bacilli as visualized under the light-emitting diode fluorescence microscope after fluorescein di-acetate staining, and 109 positive cases were detected through Löwenstein–Jensen culture. The samples were further tested to survey the drug resistance both by slide drug susceptibility test (DST) and by conventional DST: 94 MDR-TB cases were detected within 10 days through the slide DST, whereas 82 cases were observed through the conventional DST, requiring about 3 months. Because the rapidity, sensitivity and accuracy of the slide DST method were found to be comparatively satisfactory when compared to the conventional DST method; we recommend the slide DST method as the standard diagnostic tool in perspective of Bangladesh for the detection of MDR-TB.

Keywords Multidrug-resistant tuberculosis (MDR-TB) · Slide drug susceptibility test (DST) · Fluorescein di-acetate staining (FDA) staining

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Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, stands as one of the lethal health threats in Bangladesh with more than 350,000 new cases and around 70,000 deaths per year [1, 2]. The unremitting rise of multidrug resistance (MDR), which is associated with patients missing doses of medication or failing to complete their treatment, largely accounts for such a scenario [3–6]. More than 400,000 MDR-TB cases are known to emerge every year, of which around 50 % of the cases are detected in previously treated TB patients [7]. Emergence of drug-resistant *M. tuberculosis* strains is greatly complicating TB control efforts in many countries, including Bangladesh, where MDR cases have been detected in 2.2 % of the new TB cases and 14.7 % of previously treated TB cases [8]. However, curing of MDR-TB patients is difficult, as they carry strain resistance to most of the efficient anti-tuberculosis drugs [9].

To manage the TB situation in Bangladesh, the National TB Control Program (NTP) began implementing Directly Observed Therapy, Short course (DOTS), which is the internationally recommended strategy for TB control in 1993. DOTS coverage reached 100 % in 2006 and remained at that level in 2007 [1]. However, no national surveillance data on such drug resistance in Bangladesh have been available, and hence the control of MDR-TB is still substandard.

The susceptibility of *M. tuberculosis* to a specific drug is typically determined by growing the bacteria in or on media containing that drug. Commonly, the agar (LJ culture) and liquid culture (e.g., nitrate reduction assay) proportion methods are used for susceptibility testing of *M. tuberculosis* [10, 11]. Because of the slow growth of the bacteria and the requirement for isolation before drug susceptibility testing, the agar proportion method usually

requires 6–8 weeks to achieve results whereas the liquid culture methods require 4–5 weeks [12, 13].

Therefore, early detection of resistance has been essential to improve our overall TB situation. *M. tuberculosis* can be detected more rapidly on liquid media by slide culture, where it grows as a micro-colony [14]. Based on this observation, a new and relatively inexpensive method, known as the slide drug susceptibility test (DST) was first evaluated in Bangladesh by Damien Foundation; it permitted the detection of *M. tuberculosis* among true treatment failure TB patients within 10 days [14, 15]. However, the routine diagnosis of MDR cases through this rapid method compared to the currently used Löwenstein–Jensen (L-J) culture DST method remains as only theoretical knowledge and is practically unexercised in this country until now, even though the latter means of diagnosis requires a relatively longer stretch of 3 months. Nevertheless, the morbidity and mortality caused by undiagnosed MDR-TB as well as for the escalating treatment failure cases is still kept obscure. Thus, to address the growing threats of overall TB situation in Bangladesh, the feasibility of the slide DST method for the rapid diagnosis of MDR-TB needs to be extensively mapped out immediately.

Along these lines, we substantially compared the method efficacy between the slide DST method, a known (introduced by Salim et al. [15]) but unpracticed diagnostic technique, with that of the conventional DST. We observed the resistance pattern of the isolates by both the slide DST and the conventional DST, calculated the rate of detection of MDR-TB through these methods, and finally measured the performance of the slide DST method compared to the conventional DST. In agreement with the results of comparative assessment from our study, we endorse the slide DST method as a regular approach with the objective of efficient control over the true treatment failure cases.

Materials and methods

Settings

The study was carried out at National Tuberculosis Reference Laboratory (NTRL), National Institute of Diseases of Chest and Hospital (NIDCH), Bangladesh, where the patients were enrolled for this study. NTRL has been certified by the Supranational Reference Laboratory (SRL), Antwerp, Belgium, and is under regular supervision by that organization.

Study population

A total of 130 Ziehl–Neelsen (Z-N) smear-positive patients (recommended by physicians) were assigned to one of four categories: category I, failure (a patient who, during treatment under the category I regimen, remained smear positive or became smear positive again at 5 months or more after the start of treatment; $n = 41$), relapse (a previously treated patient who has been declared "cured" or "treatment completed" and is diagnosed again with smear- or culture positive tuberculosis; $n = 16$); category II, failure (a patient who, during treatment under the category II regimen, remained smear positive or became smear positive again at 5 months or more after initiation of the treatment, $n = 66$), and return after default category I and II (a patient who returns to the treatment after completion of at least 1 month of treatment and with a positive bacteriology, following interruption of treatment for 2 or more months, $n = 7$). They were then enrolled for this study from April 2011 to April 2012. Patients were admitted into NIDCH for a retreatment regimen and were monitored by the DOTS plus program. They were also examined for drug resistance within the time period indicated. It should be mentioned that Category I treatment includes the "intensive phase" where isoniazid, rifampicin, pyrazinamide, and ethambutol are administered daily for 2 months; followed by the the "continuous phase," whereby isoniazid and rifampicin are administered daily for 4 months. Category II treatment includes daily administration of streptomycin, isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months; followed by a 5-monthd continuous-phase administration of rifampicin, isoniazid, and ethambutol.

Ethical approval

Ethical approval was received from the administrative authority of NTRL, NIDCH, under nthe ational tuberculosis control program (NTP). NTRL is allowed to perform the operational results related to tuberculosis.

Collection of samples and microscopic examination for screening of acid-fast bacilli

Fresh morning sputum samples were collected on 2 consecutive days. Following the standard methodology, the direct fresh sputum sample was then used for Z-N staining [1, 15–18], fluorescein diacetate (FDA) staining [15, 19], and also for slide DST. Samples had to be decontaminated and concentrated before application to the L-J culture, followed by the conventional DST.

Slide DST

FDA-positive fresh sputum was used for this test. The media for slide DST were prepared by using Middle Brook 7H9 broth: 4.7 g broth base and 2 ml glycerine were dissolved, making the final volume 900 ml with distilled