Bangladesh J Med Microbiol 2011; 05 (02): 06-10
Bangladesh Society of Medical Microbiologists

Original Article

A rapid Drug Susceptibility Test (DST) for detection of Multi-Drug Resistant (MDR) *Mycobacterium tuberculosis* from direct sputum sample in Category II failure Tuberculosis patients.

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Abstract

This study was designed for early detection of drug resistance in Category II failure tuberculosis (TB) patients and to introduce a simple method to detect multi-drug resistant *Mycobacterium tuberculosis* (M.TB) from direct sputum sample. Total one hundred Ziehl-Neelsen (Z-N) staining positive Category II failure TB patients were enrolled in this study. Culture and drug susceptibility was done by slide and conventional method, among which 90 samples were positive by slide culture and 87 samples were positive by conventional culture. Susceptibility test by slide method showed 85 (94.44%) resistance to one or more anti -TB drugs. Resistance to Isoniazide (INH), Rifampicin (RMP), Ofloxacin (OFX) and Kanamycin (KA) was 94.44%, 84.44%, 31.11% and 4.44% respectively. By this method 80% isolates were detected as Multi-drug resistant (MDR) M.TB and 4.44% isolates were detected as Extended drug resistant (XDR) M.TB. Susceptibility results by slide DST demonstrated good concordance with conventional DST methods. Statistical analysis showed that, Sensitivity of slide DST method was 98.8%. Susceptibility results were available much faster by slide DST method (12.5±0.5 days) compared to that by conventional DST (60.4±5.9 days). Rate of contamination was also much lower by slide DST method than conventional culture (0.18% Vs 3.25%). The present study reflected that the slide DST method could be applied as rapid diagnostic tool to detect drug resistance among Category II failure TB cases, which is essential for applying appropriate therapy.

Key Words: Slide DST, MDR-TB, XDR-TB, Category II failure Tuberculosis.

Introduction

Tuberculosis is a major health problem in Bangladesh. In 2007 Bangladesh ranked 6th on the list of 22 highest tuberculosis (TB) burden countries in the world [1]. Multi-drug resistant TB (MDR) is caused by mycobacteria that are resistant to at least Isoniazide (INH), Rifampicin (RMP) which are most effective anti-TB drugs. MDR-TB results from either primary infection with resistant bacteria or may develop in the course of a patient's treatment[2]. In 2008, worldwide there were emergence of an estimated 4, 40,000 MDR-TB cases and 1, 50,000 deaths from MDR-TB. It was estimated that in 2009, 3.3% of all new cases had MDR-TB [3]. The countries that rank first to fifth rank of total number of MDR-TB cases are India (1, 31,000), China (1, 12,000), The Russian Federation (43,000) South Africa (16,000) and Bangladesh (15,000) by the end of the 2008[4]. As per National tuberculosis Control Program (NTP) report (2009), it is estimated that MDR-TB rate in Bangladesh is 1.4% among new cases and 20% among previously treated cases[1]. Treating of MDR-TB patients is difficult, as they carry strain resistant to the most efficient anti-tuberculosis drug**,** and their may be chance of development of extended drug resistance tuberculosis (XDR-TB)[5]. XDR-TB is a form of TB caused by mycobacteria that are resistant to INH and RMP (i.e. MDR-TB) with any Fluoroquinolone (FQ) and any of the second-line anti-TB injectable drugs (amikacin, kanamycin or capreomycin)[2]. Total 58 countries and territories reported at

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least one case of XDR-TB[2]. Globally estimated XDR-TB is 50,000 and death in XDR-TB is 30,000. 3 cases of XDR are reported in Bangladesh [4].

As there is possibility of drug resistance in tuberculosis, so long term treatments are given. There are several treatment categories. Category I treatment is given in case of newly diagnosed tuberculosis patients. Category II treatment is given to those patients who are previously treated with anti-TB drug for more than one month, relapse cases, category I failure, and patients with history of drug default. Treatment duration is eight (8) months. If any patient remains smear positive after 5th month or later during treatment of category II, that patient is called category II failure. These patients will be treated with category IV regimens which are mainly second line drug[6].

Most of the developing countries use solid egg based media such as Lowenstein-Jensen (L-J) and Ogawa media for mycobacterium culture and drug susceptibility test because of lower cost[7]. But it takes about 3 to 8 weeks for culture and another 4 to 6 weeks for drug susceptibility test[8]. Fully automated commercial system such as the Bactec Mycobacterial Growth Indicator Tube (MGIT960) have proved their reliability in the rapid detection of resistance of first and second line drugs, with availability of results within 10 days. But they require expensive equipments that are not easily available or suitable for resource limited countries [9].

Mycobacterium tuberculosis is detected more rapidly on liquid media by slide culture, where it grows as micro-colony. Based on this observation, a new efficient, reliable and inexpensive method, known as slide DST has been developed that permits detection of *Mycobacterium tuberculosis* as well as determination of drug susceptibility within 10 days[10]. The slide DST method may be a suitable system for early and rapid diagnosis of drug resistant TB as well as MDR-TB/XDR-TB in poor setting, requiring minimal equipment. With good contamination control and sufficient experience, a reasonably accurate MDR-TB diagnosis can be made in smear-positive cases [11].

The emergences of MDR-TB and XDR-TB have stressed the need for improved technologies for rapid detection of drug resistance in TB[12]. It has become a necessity to develop simple, inexpensive and rapid method for detection and drug susceptibility test of such suspected resistance strains. Laboratories must develop the capacity to perform DST of first line and second line drugs to detect MDR and XDR-TB using rapid methods[13].

The objective of this study is early detection of drug resistance among Category II failure TB patients and evaluation of a simple technique.

Materials & Methods:

A total 100 Z-N positive category II treatment failure tuberculosis patients of different age and sex were enrolled in this study. Sample collection and laboratory works were done in the National Tuberculosis Referral Laboratory (NTRL), National Institute of Disease of Chest and Hospital (NIDCH), during the period of January to December 2010. Culture and DST was performed by slide and conventional method.

Conventional culture and DST: Were done as per standard operating procedure on L-J media by proportion method [14].

Slide culture and DST: This method was used to determine drug susceptibility of *M. tuberculosis* to Isoniazide (INH), Rifampicin (RMP), Ofloxacin (OFX) and Kanamycin (KA), Para nitro-benzoic acid (PNB) was used for identification of *M. tuberculosis*. The study adopted the method described by Hamid *et al*., using 7H9 liquid media with albumin dextrose catalase (ADC) growth supplements, Polymyxin B, Ticarcillin, Trimethoprim, Amphotericin B (MAST tablet contain, 1 tab/ 500 medium) were added to control contamination, and anti-TB drugs. For each sample 12 sputum smears (three for control culture and eight for different drug in two different concentrations and one for PNB) were made from direct sputum sample on one end of autoclaved slides, and dipped in the McCartney bottle containing drug and drug free media. Incubated at 37^{o} C for 12 days. After 12 days, the bottle was removed from incubator to the safety cabinet. A forceps was used to remove the slide from the bottle and the slide was dipped in a container containing rectified spirit, removed them after five minutes and were let them dry on a brown paper. Slides were fixed in the flame of spirit lamp and stained by Z-N staining. The smear was read at 100X for micro colonies. Any number of well-developed colonies with cording in presence of a drug was interpreted as resistance (Fig:1).

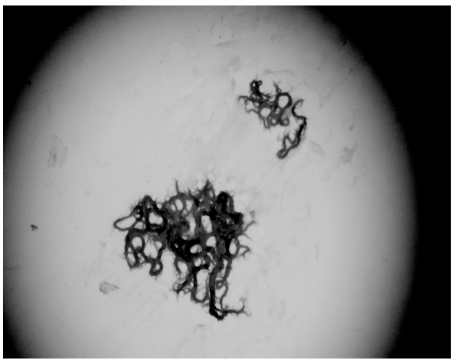

Figure 1: Micro colonies of *M. tuberculosis* showing cording appearance at 100X magnification under bright field microscope

The critical concentrations and interpretation applied during the study period had been adopted from the original publication and protocol of Damien Foundation[11].

Results

Among 100 patients 90% were culture positive by Slide method and 87% were culture positive by conventional method (Table: I).

Table I: Comparison of results Slide culture with Conventional culture in L-J media (n-100).

Slide Culture	Number	Conventional culture	
		Positive *n* (%)	Negative *n* (%)
Positive	**90**	86 (95.5)	4 (4.5)
Negative	**10**	1 (10)	9 (90)
Total	**100**	87 (87)	13 (13)

By slide DST highest number of resistance was found in INH that was 85 (94.44%) followed by RMP 76 (84.44%). MDR by slide DST was detected in 72 (80%) samples. Forty eight (53.33%) samples were resistant to two drugs (INH+RMP), 24 (26.66%) resistant to three drugs (INH+RMP+OFX), and 4 (4.44%) were found as XDR as they were resistant to four drugs. Nine (10%) samples were mono (only INH) drug resistance (Table: II).

Table II: Resistance pattern four anti-TB drugs by Slide and conventional DST.

Resistance pattern	Anti-TB drugs	Slide DST	Conventional DST
Mono	Only INH	09 (10.00)	10 (11.49)
	Only RMP	00 (00.00)	00 (00.00)
	Only OFX	00 (00.00)	00 (00.00)
	INH+ RMP	48 (53.33)	46 (52.87)
Multi	INH+RMP+OFX	24 (26.66)	23 (26.44)
	**INH+RMP+OFX+KA	04 (04.44)	03 (03.45)
	No resistance	05 (05.55)	05 (05.74)
	Total	**90 (100))**	**87 (100)**

Statistical analysis showed that sensitivity of slide DST method was 98.8%. Detection time was much shorter in case of slide DST. Conventional method susceptibility results obtained in 50-70 days with mean of 60.0±5.9 days and slide DST results obtained in 12-13 days with mean of 12.5±0.5 days (Table: III). Rate of contamination is higher in conventional culture that was 3.25% in comparison to slide DST method that was 0.18% (Table: III).

Table III: Comparison of conventional DST and slide DST.

Method	Susceptibility detection time	Rate of contamination
Conventional DST	(50-70) days	3.45%
Slide DST	(12-13) days	0.18%

Discussion:

Early drug susceptibility test is important in tuberculosis patients. In case of category II failure patients it is more important because there are chances of development of MDR-TB. In Bangladesh, drug susceptibility is done on solid media as it is low cost and it takes 2-3 months time. The present study was designed to rapid detection of drug resistances pattern in Category II failure TB patients and to introduce a simple and rapid method.

In slide DST, 85 (94.44%) cases were found to have resistance to one or more drugs. Resistance of INH was highest (94.44%) followed by RMP (84.44%). Other authors found 86% and 76% resistance to INH among Category II failure patients[15, 16]. By slide DST, 72 cases (80%) were detected as multi-drug resistance. Other authors found 83% and 87% MDR-TB, respectively among Category II failure patients by[15,16]. The probable causes of above finding may be due to treatment with an inadequate drug regimen or re-infection while under treatment[17]. The sensitivity of slide DST was 98.8%. Another author found 100% sensitivity and 62% specificity[11]. Cause of lower specificity may be due to more contamination and they are suggesting using PANTA.

Thirteen bottles were contaminated in conventional culture in which 9 samples were positive in 2nd sample and 4 samples were negative in culture. In slide DST only 2 bottles of control of separate samples were contaminated but growth was present in other 2 control bottles. Rate of contamination was higher in conventional culture (3.25%). Tortoli *et al*., found 11% contamination in their study by conventional method, which is not similar with present study[18]. This may be due to use of four sets of culture media for each patient. If any one was contaminated, result was interpreted from the

other bottles. In the present study lower rate of contamination (0.18%) was found in slide DST. This may be due to use of MAST selectatab as decontaminant. Hamid *et al.* was found 7.4% contamination by slide DST in their study[11]. Possible explanation of these authors findings are the use of penicillin instead of PANTA or MAST tablet.

Drug susceptibility detection time by slide DST method in the present study was 12.5 days, and it correlates with other studies - 10 days by Hamid *et al*[11], 8 days by Dickinson *et al.*[10] Whereas, detection time by conventional DST was 60.4±5.9 days. Accordingly, slide DST provides good opportunity for rapid identification of MDR strain. This early information is of great advantage in clinical settings to choose an appropriate drug regimen.

The limitation of slide DST is that, although it is cheaper than automated culture methods, it requires costly media, growth and antibiotic supplements, which are not commonly available in field level laboratories in the developing countries. Another limitation of slide DST is, only microscopically positive sputum samples could be tested. Quality assurance is also a challenge, as control strains cannot be used. Slide DST is qualitative tests in which the observer confirms resistance by visualizing growth under microscope; where any well developed micro colony in presence of drug interprets as resistance to that drug. In slide DST there are no discrete colonies to count and a proportion cannot be calculated. As a result slide DST might over diagnose resistance, that is, isolates read as susceptible by reference method.

Slide DST can provide good opportunity for rapid detection of drug resistance TB including MDR/XDR strain from direct sample within 12 days. This method can be implemented as rapid diagnostic tool to detect drug resistance among Category II failure TB cases.

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