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Original Article

XDR and MDR tuberculosis from a tertiary chest hospital in Bangladesh.

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Abstract

The present article provides an overview of the new diagnostic options available for diagnosis of drug resistant tuberculosis, which include liquid based culture and susceptibility tests. To effectively address the threats of drug resistant tuberculosis, global initiatives are required to scale-up culture and drug susceptibility testing capacities. In parallel efforts are needed to expand the use of new technologies for rapid diagnosis of drug resistance.The study was carried out at National Institute of Diseases of Chest and Hospital (NIDCH), Mohakhali, Dhaka, which is the only tertiary- level chest disease hospital for the treatment of referred patients from all over the country. One morning sputum sample was taken from each of the 50 suspected new pulmonary tuberculosis patients and 50 samples from previously treated group included patients who did not complete the full regimen or those who did not respond to antibiotic treatment. All samples were cultured on sula liquid media for 12 days. Sensitivity tests against isoniazid (INH), rifampicin (RMP), streptomycin (SM) and ethambutol (EMB), gatifloxacin (G),kanamycin (K) and ofloxacin (OF) were done by slide culture method. Sensitivity test against P-nitro benzoic acid (PNB) were also done to differentiate typical Mycobacterium from atypical Mycobacterium.Among the 50 isolated new cases 98% were sensitive to isoniazid, rifampicin and streptomycin. Regarding resistant pattern for MDR-TB to second line drugs, 2% were resistant to kanamycin and 10% to ofloxacin.One MDR-TB which was resistant to kanamycin was also resistant to ofloxacin. Hence it was identified as extensively-drug resistant tuberculosis (XDR-TB).No atypical Mycobacteria were detected by PNB.

Key words: MDR-TB, XDR-TB

Introduction:

The impact of tuberculosis (TB) can be devastating even today, especially in developing countries suffering from high burdens of both TB and human immunodeficiency virus (HIV) infections. In 2009 there were 9.4 million new cases of TB globally, causing 1.7 million deaths[1]. Drug resistance has enabled it to spread with a vengeance. The prevalence of multi-drug resistant tuberculosis (MDR-TB) and extensively-drug resistant tuberculosis (XDR-TB) are increasing throughout the world both among new tuberculosis cases as well as among previously treated ones[2]. Fortunately, the past few years have seen an unprecedented level of funding and activity focused on the development of new tools for diagnosis of drug resistant tuberculosis. This should go a long way in helping us arrest the spread of the disease.

Multidrug-resistant tuberculosis (MDR-TB) is a form of tuberculosis that is resistant to two or more of the primary drugs (isoniazid and rifampicin) used for the treatment of tuberculosis[3]. The World Health Organization estimates that there were half a million cases of MDR- TB worldwide in 2007, the highest ever reported. These cases are not spread proportionately across the globe, as only 27 countries account for 85 percent of all MDR TB cases. Unfortunately, only a small portion of MDR-TB cases are treated properly each year; about 1 percent (3,700) in 2007[4].

XDR-TB is defined as TB caused by a strain of M. tuberculosis that is resistant to rifampicin and isoniazid, as well as to any member of the quinolone family and at least one of the second-line anti-tuberculous injectable drugs i.e., Kanamycin, Capreomycin, or Amikacin. XDR-TB was first

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described in 2006. Since then, these have been documented in six continents and 55 countries[4]. Treatment outcomes are significantly worse for patients with XDR-TB, compared to patients with drug-susceptible TB or MDR TB5. In the first recognised outbreak of XDR-TB, among the 53 patients in KwaZulu-Natal, South Africa, who were co-infected with human immunodeficiency virus (HIV), survived for an average of 16 days, the mortality rate was 98%6. XDR-TB raises concerns of a future tuberculosis epidemic with restricted treatment options, and jeopardises the major gains made in tuberculosis control.

The success of treatment of MDR-TB depends upon how quickly a case of TB is identified as drug resistant and whether an effective drug therapy is available. The second-line drugs used in cases of MDR -TB are often less effective and more likely to cause side effects[7]. Tests to determine the resistance of a particular strain to various drugs usually take several weeks to complete. During the delay, the patient may be treated with a drug regimen that is ineffective. Once a strain's drug resistance is known, an effective drug regimen must be identified and begun[8].

Mycobacterium tuberculosis is an extremely slow growing organism. Using standardised drug susceptibility testing (DST) with conventional methods, 8 to 12 weeks are required to identify drug resistant tuberculosis. As the results are delayed, inappropriate choice of treatment regimen may result in death mainly in case of XDR-TB (especially in HIV co-infected). In addition, delayed diagnosis of drug resistance results in inadequate treatment, which may generate additional drug resistance and continued transmission in community.Microscopic observation of drug susceptibility assay is based on characteristic cord formation of M. tuberculosis that can be visualised microscopically ('strings and tangles' appearance) in liquid medium with or without antimicrobial drugs (for DST) [9]. The test sensitivity is better than traditional methods. Besides, it is cheap, simple and fairly accurate[10].

Materials And Methods:

Procedure of sputum collection

One spot sputum sample was collected in a container. On the initial hospital visit, the patient was provided a clean, dry, wide-neck, leak-proof container and requested him or her to cough deeply to produce a sputum specimen (3-5 ml volume)

Slide DST:

For slide DST, the method using Sula liquid medium with 10% locally collected goat serum was adopted as described by Dissmann. In this media positive or negative growth as well as sensitivity pattern of first and second line drugs can be seen in the same settings. Eleven sputum smears were made on one end of a longitudinally half autoclaved slide. This then dried in safety cabinet and placed individually in sterile, heavy glass 28 ml universal bottles containing 7 ml medium. For each sample, two growth controls (C1, C2), one half slides for RMP (1.0µg/ml), one for INH (1µg/ml), one for EMB (5.0µg/ml), one for SM=2µg/ml, one for K (10µg/ml), one for G (8.0µg/ml), one for OF (0.5µg/ml), one for PNB (P=500µg/ml) and one nicotinamide (N=40µg/ml). The bottles were incubated at 36°C for 12 days. After 12 days the bottles were removed from incubator and placed in a safety cabinet. After opening the bottle with a forcep, the slides were removed from the bottle. The slides were briefly dipped in a universal container containing rectified spirit and dried on a brown paper in the safety cabinet. The used spirit was discarded in a jar containing 5% phenol. After air dry, the smears were fixed in the flame of spirit lamp. The slides were arranged in a slide- rack placed in a staining jar and sufficient 1% carbolfuchsin was used to immerse the jar for 30 mins and then the other steps of Z-N staining was continued[11].

Results

Table-I: Distribution of slide drug susceptibility test (DST)

Slide DST	TB cases		Total
	Treatment failure	New cases	
INH 1µg/ml			
Sensitive	0	49 (98)	49 (49)
Resistant	*1+49 (100)	1 (2)	51 (51)
RMP 1µg/ml			
Sensitive	0	49 (98)	49 (49)
Resistant	*1+49 (100)	1 (2)	51 (51)
EMB 5µg/ml			
Sensitive	11 (22)	50 (100)	61 (61)
Resistant	*1+38 (78)	0	39 (39)
SM 2µg/ml			
Sensitive	8 (16)	49 (98)	57 (57)
Resistant	*1+41 (84)	1 (2)	43 (43)
G 8µg/ml			
Sensitive	50 (100)	50 (100)	100 (100)
Resistant	-	-	-
K 10µg/ml			
Sensitive	49 (98)	50 (100)	99 (99)
Resistant	*1(2)	0	1 (1)
OF 0.5 µg/ml			
Sensitive	45 (90)	45 (90)	90 (90)
Resistant	*1+4 (10)	5 (10)	10 (10)
NICO 40 µg/ml			
Sensitive	48 (96)	50 (100)	98(98)
Resistant	2 (4)	-	2 (2)

*XDR-TB

Figures within parentheses indicate percentage

Among the Mycobacteria isolated from new cases, 49(98%) were sensitive to isoniazid, rifampicin and streptomycin;

50(100%) to gatifloxacin, kanamycin and nicotinamide (Table I).Among the 50 MDR-TB cases, all were resistant to isoniazid and rifampicin, 39(78%) to ethambutol, 42(84%) to streptomycin, 2(4%) to nicotinamide, 1(2%) to kanamycin and 5(10%) to ofloxacin.

Discussion:

The diagnosis is an important tool for TB control because it allows establishing the correct treatment and the required further control practices to break down the transmission chain of the bacilli.In this study, PNB was used in drug susceptibility test to differentiate typical and atypical Mycobacteria. All Mycobacteria isolated from TB cases showed sensitivity to PNB that indicates there were no atypical Mycobacteria. No Mycobacterium isolated from new cases showed resistance to both isoniazid and rifampicin. From these findings it can be assumed that adequate treatment with proper antitubercular drugs will reduce the chance of emergence of MDR-TB.

Mycobacteria isolated from MDR-TB, 49 (98%) were sensitive and one (2%) was resistant to kanamycin (10µg/ml). All Mycobacteria including MDR-TB and new cases were sensitive gatifloxacin (8µg/ml). Among the MDR-TB, 48 (96%) were sensitive to nicotinamide and in new cases all were sensitive to nicotinamide (40µg/ml). Hamid et al. (2002) reported similar results on doing the sensitivity test in liquid media and found that the prevalence of true resistant was about 50-68% for isoniazid, 50-71% for rifampicin, 21-30% for ethambutol, 40-50% for streptomycin. One MDR-TB which was resistant to kanamycin was also resistant to ofloxacin. These Mycobacteria was identified as XDR-TB. On literature survey it seems that this is the first case report from Bangladesh. However, this is very alarming findings as this may be the tip of the ice-berg. In a poor resource country like Bangladesh, the spread of XDR-TB will be an additional burden to the health system.Conventional laboratorial methods for diagnosis of TB are sputum microscopy and culture in Lowenstein- Jensen media[12]. However these methologies present low sensitivity or take a long time to achieve results. Even though there are several new methodologies with higher specificity and or ssensitivity able to provide earlier results than than the conventional methodologies, the implementation of these alternative methods in the routine of the majority of the laboratories around the world is very difficult because of their high cost and complexity[13].

The development of new, inexpensive and more sensitive method for TB diagnosis able to provide earlier results than the conventional methods is a priority for TB control. The detection of microcolonies has been described as an alternative method for the diagnosis of TB[14]. This study evaluated the sensitivity and time for detection of positive cultures with the microcolony methods.Although present worldwide, the incidence of TB is higher in poor regions, where tools for controlling this diseases are necessary. The slide culture method presents advantages, such as simple,execution, easy interpretation and faster results. Results of this work confirm that thiis method is a reliable alternative method for the diagnosis of TB.

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