First Line Anti-Tubercular Drug Resistance Pattern of *Mycobacterium Tuberculosis* Isolated From Specialized Hospitals of Dhaka City

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Abstract

The present study was undertaken to determine the drug resistance pattern of *M. tuberculosis* isolated from 225 pulmonary and 45 extrapulmonary tuberculosis cases. The samples were cultured on Lowenstein Jensen (L-J) media for isolation of *M. tuberculosis*. Drug resistance to first line anti tubercular drugs-namely isoniazid (INH), rifampicin (RIF), Ethambutol (ETH) and streptomycin (SM) were determined by indirect proportion method. The overall drug resistance of *M. tuberculosis* was 53.6% to any of the first line anti tubercular drugs. Rate of multi drug resistant tuberculosis (MDR-TB) among the untreated cases was 4.2%, while it was 36.0% in previously treated cases. It was found that 83.3% rifampicin resistant *M. tuberculosis* was cross resistant to one or more of other first line anti-tubercular drugs, while cross resistance of INH, ETH and SM resistant isolates was much low. The present study revealed that high level of drug resistance exists to individual anti tubercular drugs and MDR-TB is an emerging problem, particularly in treated cases. Rifampicin resistance could be used as a surrogate marker for drug resistance to other first line anti tubercular drugs.

Ibrahim Med. Coll. J. 2014; 8(2): 41-46

Introduction

Tuberculosis (TB), caused by *M. tuberculosis* (MTB) is one of the important cause of morbidity and mortality in many countries of the world. The incidence of the disease has remained high in most of the developing countries. In 2009, there were an estimated 9.4 million TB cases and 1.3 million deaths. Most of the estimated number of cases in 2009 occurred in Asia (55%), Africa (30%), the Eastern Mediterranean (7%), Europe (4%), and the region of the Americas (3%).[1]

In Bangladesh, TB remains a major public health problem. Over 300,000 new cases of TB and 70,000 deaths are estimated to occur per year in Bangladesh and the country ranks 6th out of the 22 highest TB burden countries of the world.[2] The estimated incidence and prevalence rate of all forms of TB were 223 and 387 per 100,000 population respectively. The estimated death rate was 45 per 100,000 population.

Drug resistant TB is widespread and is now a threat to TB control program in many countries including Bangladesh. In Bangladesh, resistant to INH, SM, ETH and RIF ranged from 15.8-23.0%, 6.9-18.0%, 2.9-10%, 2.0-10.9% respectively.[3,4]

Globally the median prevalence of drug resistance to any drug in untreated cases was the highest (19.8%) in South East Asia (SEA) followed by Western Pacific (11.4%) and Europe (8.4%). The median prevalence of drug resistance to any drug in treated cases was the highest (63.3%) in the Eastern Mediterranean followed by SEA (39.9%) and (in Europe (15.9%). The rate of MDR- TB ranged from 4.7%-48.3% in above regions.[5]

The pattern of drug resistance changes continuously over time in a given area and with the use of anti-TB drugs. Therefore, it is important to determine the rate of drug resistance at a certain interval. So, monitoring of drug resistance pattern, early accurate diagnosis and initiating prompt treatment have been the mainstay to interrupt the transmission and control of TB.

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The present study was undertaken to determine the rate of drug resistance of MTB to first line anti-tubercular agents in patients attending tertiary care hospitals of Dhaka city. The study also investigated the concomitant resistance of MTB among rifampicin resistant MTB.

Materials and Methods

Two categories of patients namely, suspected pulmonary and extra pulmonary tuberculosis cases were included. Sputum and lymph node (LN) aspirates were collected from the pulmonary and extra pulmonary TB cases respectively. Sputum was collected from a total of 255 suspected TB patients who attended the out patient department (OPD) of Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Tuberculosis Control and Training Institute (TCATI), Chankharpool, outdoor and admitted patients of National Institute of Disease of Chest and Hospital (NIDCH), Mohakhali, Dhaka. LN aspirates were collected from 45 patients of suspected cervical and axillary tubercular lymphadenitis attending the OPD of Dhaka Medical College Hospital (DMCH). The study was carried out during the period of April 2005 to September 2010.

Sample collection and processing

The early morning sputum samples were collected in clean, sterile wide mouthed container closed with lid. The quantity of sputum collected from each patient was 2 – 5 ml. The LN aspirates were collected aseptically in 50 ml of sterile Falcon tubes containing 3 ml sterile distilled water in each container. The containers were labeled with patient's name, identification number and date. The samples were brought to the department of Microbiology, BIRDEM, Dhaka as soon as possible, where necessary laboratory tests were done after processing the samples in Class 2 bio-safety cabinet.

All the samples (sputum and LN aspirates) were digested and decontaminated of other bacteria by N-Acetyl-L- Cystine (NALC) + 4% Sodium Hydroxide method as described by Kent and Kubica. [6]

The processed products of the samples were kept in 3 different eppendorf tubes for: a) Ziehl-Neelsen (ZN) stain, b) culture of mycobacteria on Lowenstein Jensen (L-J) media and c) rapid detection of mycobacteria by PCR method. Smear was stained by ZN method for the detection of acid fast bacilli (AFB). Culture was done by inoculating it on L-J media and incubating it at 37^0C for isolation of mycobacterium. The culture bottles were examined weekly for 8 weeks for the evidence of growth. On appearance of visible colonies, the colony morphology, rate of growth and pigment production were noted. The growth of M. *tuberculosis* was identified by staining of colonies with ZN stain and confirmed by necessary biochemical tests.[6]

Drug susceptibility test

Drug susceptibility to isoniazid (INH), rifampicin (RIF), streptomycin (SM) and ethambutol (ETH) was determined by indirect proportion method.[6] The drugs used for susceptibility test were obtained from Aventis, Bangladesh except streptomycin which was obtained from Opsonin Chemical Industries Ltd, Dhaka with proper label mentioning manufacturing and expiry date. The potency of each antibiotic was verified by the reference strain $H_{37}Rv$.

Interpretation of Culture

The number of colonies on control and drug containing media were counted and the percentage of the resistant organisms was calculated as follows:

(Number of colonies on drug containing media / Number of colonies on control media) X100 = % of resistant

If the percentage of resistant organism was 1% or more, then the isolate was considered resistant to the specific drug. A set of tubes with and without drugs were incubated with reference strain *M. tuberculosis* $H_{37}Rv$ as a quality control.

Results

A total of 300 suspected cases of tuberculosis were included in this study. Sputum was collected from 255 clinically suspected pulmonary TB cases and lymph node aspirate was collected from 45 extra-pulmonary TB cases to determine the rate of anti mycobacterial drug susceptibility. The patients were selected from BIRDEM, NIDCH, TCATI and DMCH.

Table-1 shows the results of culture of the study samples. Out of the total 300 samples, sputum sample

Table-1: *Results of culture of study samples*

Specimen	Total Number	Culture of *Mycobacterium*		Samples Contaminated No. (%)
		Positive No. (%)	Negative No. (%)	
Sputum	255	180 (70.59)	40 (15.69)	35 (13.72)
LN aspirate	45	20 (44.45)	15 (33.33)	10 (22.22)
Total	300	200 (66.67)	55 (18.33)	45 (15.00)

Table-2: *Species distribution of culture positive Mycobacteria in sputum and LN aspirates*

Samples	Total culture positive	*Mycobacterium* sp	
		M. tuberculosis	MOTT
Sputum	180	176 (97.8)	4 (2.2)
LN aspirates	20	16 (80.0)	4 (20.0)
Total	200	192 (96.00)	8 (4.00)

MOTT = Mycobacterium other than tuberculosis

was 255 of which 180 (70.59%) were culture positive, 40 (15.69%) were culture negative and 35 (13.72%) became contaminated. Among the 45 lymph node aspirates 20 (44.45%) were culture positive, 15 (33.33%) were culture negative and 10 (22.22%) became contaminated. Table-2 shows the species distribution of culture positive mycobacteria in sputum and LN aspirates. Out of 180 culture positive isolates from sputum 176 (97.8%) were *M. tuberculosis* and 4 (2.2%) were mycobacterium other than tuberculosis (MOTT). Out of 20 isolates from lymph node aspirates 16 (80.0%) were *M. tuberculosis* and 4 (20.0%) were MOTT.

Overall susceptibility pattern of *M. tuberculosis* and MOTT to first line anti-TB drugs are depicted in Table-3. Out of 192 *M. tuberculosis* isolates 89 (46.35%) were sensitive to all of the four first line anti-TB drugs and 103 (53.65%) were resistant to any of the four first line

Table-3: *Overall susceptibility pattern of M. tuberculosis and MOTT to first line anti-TB drugs*

Organism	Total Isolates	Resistant to any drug No. (%)	Sensitive No. (%)
MTB	192	103 (53.65)	89 (46.35)
MOTT	08	08 (100)	00

Note: *MTB: M. tuberculosis; MOTT = Mycobacterium other than tuberculosis; Sensitive means sensitive to all drug (INH, RIF, ETH, SM).*

Table-4: *Rate of drug resistance of M. tuberculosis isolated from untreated and treated tuberculosis cases*

Category of cases	Total cases	Overall resistant to				Resistant to any drug
		INH No. (%)	RIF No. (%)	ETH No. (%)	SM No. (%)	No. (%)
Untreated cases	167	37 (22.15)	16 (9.58)	22 (13.17)	37 (22.15)	78 (46.71)
Previously treated cases	25	13 (52.0)	14 (56.0)	17 (68.0)	13 (68.0)	25 (100)

Note: *INH- isoniazid, RIF- rifampicin, ETH- ethambutol, SM-streptomycin*

anti-TB drugs. In case of the MOTT, all 8 (100%) were resistant to any of the first line anti-TB drugs.

Out of the total 192 *M. tuberculosis* isolates, 167 were untreated and 25 were treated cases (Table 4). Among the 167 untreated cases 78 (46.71%) were resistant to any of the four first line anti-TB drugs and overall drug resistance pattern was INH 37 (22.15%), RIF 16 (9.58%), ETH 22 (13.17%), and SM 37 (22.15%). Among the treated cases all 25 (100%) were resistant to any drug and overall drug resistance pattern were INH 13 (52.0%), RIF 14 (56.0%), ETH 17 (68.0%) and SM 13 (52.0%).

Table-5 shows resistance pattern of 167 *M. tuberculosis* isolates to 4 first line anti-TB drugs in untreated cases. Out of the total 167 isolates, 53 (31.74%) were resistant

Table-5: *Resistant pattern of M. tuberculosis to 4 first line anti-tubercular drugs isolated from untreated tuberculosis cases (n=167)*

No. of drugs	Drugs	Resistant No.	%	Total No.	%
One drug	Only INH	18	(10.78)	53	(31.74)
	Only RIF	5	(2.99)		
	Only ETH	8	(4.79)		
	Only SM	22	(13.17)		
Two drugs	*INH+RIF	1	(0.60)	19	(11.38)
	INH+SM	4	(2.40)		
	INH+ETH	8	(4.79)		
	RIF+SM	4	(2.40)		
	ETH+SM	2	(1.20)		
Three drugs	*INH+RIF+SM	2	(1.20)	3	(1.80)
	*INH+RIF+ETH	1	(0.60)		
Four drugs	*INH+RIF+ETH+SM	3	(1.80)	3	(1.80)

*Note: * indicates MDR-TB*

Table-6: *Resistance pattern of M. tuberculosis to 4 first line anti-TB drugs isolated from previously treated cases (n=25)*

No. of drugs	Name of resistant drugs	Resistant No.	Resistant %	Total No.	Total %
One drug	Only ETH	02	08		
	Only SM	02	08	04	16
	*INH+RIF	1	04		
	INH+SM	3	12		
Two drugs	INH+ETH	1	04		
	RIF+ETH	4	16	13	52
	RIF+SM	1	04		
	ETH+SM	3	12		
Three drugs	*INH + RIF+SM	1	04	1	4
Four drugs	*INH+RIF+ETH+SM	7	28	7	28

*Note: * indicates MDR-TB*

to one drug, 19 (11.38%) were resistant to two drugs, 3 (1.80%) were resistant to three drugs and 3 (1.80%) were resistant to four drugs. Table-6 shows resistance pattern of *M. tuberculosis* to four first line anti-TB drugs in previously treated cases. Out of the total 25 isolates, 4 (16.0%) were resistant to one drug, 13 (52.0%) were resistant to two drugs, 1 (4.0%) was resistant to three drugs and 7 (28.0%) were resistant to four drugs.

Table 7 shows the rate of MDR-TB in untreated and treated pulmonary TB cases. Among the untreated cases, MDR-TB was 4.2% while it was 36.0% among the treated cases. The rate was significantly higher in previously treated group. The rate of concomitant resistance pattern of RIF resistant *M. tuberculosis* to INH, ETH and SM are described in Table-8. It was observed that 83.3% RIF resistant *M. tuberculosis* isolates were resistant to other three drugs. The association of RIF resistance with resistance to other three drugs were significantly associated ($p < .05$). The concomitant resistance of INH, ETH and SM resistant

Table-7: *Rate of isolation of MDR-TB from untreated and treated pulmonary tuberculosis cases*

Categories	Total Number	MDR NO (%)
Untreated	167	7 (4.2)
Treated cases	25	9 (36.0)

M. tuberculosis to any other three drugs were 55.5-74.3% and the co-resistance was not significantly associated ($P > 0.05$).

Table-9 shows the concomitant resistance rate of *M. tuberculosis* to any three first line anti-TB drugs which were sensitive to RIF, INH, ETH or SM. Rate of resistance to three other drugs ranged from 34.78% to 43.21% among RIF, INH, ETH or SM sensitive isolates.

Discussion

The majority of the TB cases occur in developing countries with limited resources. Currently, tuberculosis control is potentially difficult worldwide due to the emergence of drug resistance to first line anti-tubercular drugs and MDR-TB.[5] The appearance of totally drug resistant tuberculosis (TDR-TB) has made the situation worse.[7]

Monitoring of drug resistance pattern, early diagnosis and initiating prompt treatment has been the mainstay to interrupt the transmission of tuberculosis. In this context, the present study was designed to determine the drug resistance pattern of mycobacterium. In the present study, about 70.0% sputum samples yielded positive culture results on L-J media. Various authors have reported similar culture positivity rate in L-J media which ranged from 59.72 to 87.2%.[8-11] However, the culture positivity rate was only 44.0% in lymph

Table-8: *Rate of concomitant resistance pattern of RIF resistant M. tubercolosis to INH, ETH and SM*

Resistant to	No	Resistant to any 3 other drugs	No. of isolates concomitantly resistant to			
			RIF	INH	ETH	SM
RIF	30	25 * (83.3) P<0.05	-	16 (53.3)	15 (50.0)	18 (60.0)
INH	50	32 ** (64.0) P>0.05	16 (32.0)	-	20 (40.0)	20 (40.0)
ETH	39	29 (74.3)	15 (38.4)	20 (51.2)	-	15 (38.4)
SM	54	30 (55.5)	18 (33.3)	20 (37.0)	15 (27.8)	-

Note: * $\div^2$ test between RIF resistant and resistant to other three drugs ($P < 0.05$); ** $\div^2$ test between INH resistant and resistant to other three drugs ($P > 0.05$); RIF-rifampicin, INH- isoniazid, ETH- etambutol, SM- streptomycin

Table-9: *Rate of concomitant resistance of RIF / INH / ETH /SM sensitive M. tuberculosis to corresponding drugs*

Sensitive to	No.	Resistant to other 3 drugs		No. (%) of isolates concomitantly resistant to			
		No.	%	RIF	INH	ETH	SM
RIF	162	70	(43.21)	—	30 (18.52)	21 (12.96)	36 (22.22)
INH	143	52	(36.36)	13 (9.09)	—	19 (13.28)	34 (23.77)
ETH	154	63	(40.90)	14 (9.09)	29 (18.83)	—	38 (24.67)
SM	138	48	(34.78)	12 (8.69)	29 (21.01)	23 (16.66)	—-

Note: INH- isoniazid, RIF- rifampicin, ETH- ethambutol, SM - streptomycin

node aspirate samples. The failure to isolate mycobacteria in about 30-56% sputum and lymph node aspirates was due to contamination of media or damage to organisms during decontamination process. Previous studies reported the contamination rate from 1.2% to 27.2%.[9-13] Therefore, the isolation rate of mycobacteria can be increased if contamination is prevented and sample processing procedure is further improved. Out of the 200 isolates of mycobacteria, 96.0% were *M. tuberculosis* and 4.0% were MOTT. Earlier, a study in Dhaka by Miah *et al*. reported 95.3% isolates as *M. tuberculosis* and 4.7% as MOTT.[3]

In the present study, 53.65% MTB isolated from untreated cases was resistant to any first line anti-tubercular drugs while the rate among previously treated cases was 100%. Previously, in the year 2000 Miah *et al*[3] from Bangladesh reported that 29.7% of *M. tuberculosis* was resistant to at least any one of the first line anti-tubercular drugs. In 2007, Rahim *et al*[4] reported the rate of resistance to any single first line anti-tubercular drug as 31% among patients attending TB clinic in Sunamganj, a district located about 250 km north east of the capital, Dhaka. Therefore, it appears that in last ten years the rate of resistance of *M. tuberculosis* has increased from 29% to 53% in the selected population of urban areas. This high rate of resistance among cases in Dhaka could be due to the fact that complicated cases are referred to Dhaka. World wide reported resistance to any anti-tubercular drugs ranged between 9.8-39.3%.[5]

The resistance pattern of first line anti-tubercular drugs observed in the present study among untreated cases was almost similar to the resistance pattern reported previously in 2000 and 2007.[3,4] Almost similar rate of resistance was observed in other neighboring countries.[14,15]

The drug resistance rate was higher in *M. tuberculosis* isolated from treated cases compared to that of untreated cases. In this study 4.2% M. *tuberculosis* isolated from untreated cases and 36.0% of *M. tuberculosis* isolated from treated cases were MDR-TB. Global prevalence of MDR-TB among untreated cases ranged from 0.4 to 1.4% and it was 4.7%-48.3% among treated cases. [5]It has been estimated that globally 3.3% of all TB cases were MDR-TB in 2009 which is closer to the findings of the present study.[16]

In the present study, out of 30 RIF resistant *M. tuberculosis*, 83.3% were also concomitantly or cross resistant to other three first line anti-tubercular drugs ($p < 0.05$; Table-8). On the other hand, of the 50 INH resistant *M. tuberculosis*, 64.0% were concomitantly or cross resistant to other three first line anti tubercular drugs ($p > 0.05$) while for ETH and SM the rate was 74.3% and 55.5% respectively. Resistance to RIF in *M. tuberculosis* occurs in a high frequency and mono resistance to RIF is rare, whereas mono resistance to INH is common.[17] It has been proposed that resistance to RIF can be used as a surrogate marker for MDR-TB as nearly 90% of the RIF resistant strains are also INH resistant.[17,18] It is to be noted that only 43.21% *M. tuberculosis* isolates which were sensitive to RIF, was concomitantly resistant to other 3 drugs (Table-9). This indicates that a sensitive *M. tuberculosis* isolates (sensitive to RIF, INH, ETH and SM) could be resistant to any of the three other first line anti-TB drugs and it could not therefore, predict that if an isolate sensitive to any single first line drug would simultaneously be sensitive to other three drugs.

The present study, therefore, revealed that high level of drug resistance exists to individual anti- tubercular drugs and MDR-TB was an emerging problem particularly in treated cases. Rifampicin resistance could be used as a surrogate marker resistance to other drugs and could obviate the necessity of doing susceptibility test with other drugs in a resource constraint situation.

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