DETECTION OF RIFAMPICIN RESISTANCE IN PULMONARY TUBERCULOSIS BY LOWENSTEIN JENSEN CULTURE METHOD

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ABSTRACT

Objective: Pulmonary Tuberculosis a transmissible disease is caused by *Mycobacterium tuberculosis* (MTB). *M.tuberculosis* is gaining resistance to first line Anti-TB drugs, thus leading to drug resistant pulmonary tuberculosis (DR-PTB). Our study attempted to determine rifampicin resistance in mycobacterium tuberculosis isolated from patients with pulmonary tuberculosis. **Study Design:** A cross sectional study.**Place and Duration**: The Department of Microbiology BMSI, JPMC Karachi from October 2015- September 2016. **Material and Methods**: The study was conducted on 220 pulmonary TB suspects (fresh and retreated) on clinical and radiological grounds. A history of contact with MDR- TB patients and previous use of anti-tuberculosis therapy were documented. All sputum samples were analyzed by the lowenstein Jensen (LJ) culture media. **Results:** Among 220 PTB cases 167 (75.90 %) were MTB positive. Out of 167, 35 (20.95%) were RIF positive. Among RIF resistant cases male to female ratio was more than 6:1 (57.14: 8.98). Primary and secondary RIF resistance were found as 4.79%, 16.16% respectively. Among secondary resistance 10.77% and 5.38% were found defaulters and relapse patients consecutively. **Conclusion:** Secondary rifampicin resistant PTB is continuously increasing in our community so detection of RIF resistance in PTB cases by culture method is essential for further stoppage of emergence of MDR-PTB.

Key words: Rifampicin, Multidrug resistant tuberculosis, Mycobacterium TB, Pulmonary TB

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INTRODUCTION:

Tuberculosis can invade any part of the body, but pulmonary tuberculosis is the most common form, accounting for 80% of all cases[1]. *Mycobacterium tuberculosis* is a human obligate aerobic bacterium and facultative intracellular parasite[2]. It produces nodules in the lungs thereby it is also known as tubercle bacillus[3].

M. tuberculosis (MTB) is a slow-growing bacterium with a doubling time of 16-22 hours[4]. MTB has strong acid resistance, and its length and width vary from 1-4 x 0.3-0.6 µm[5]. Pulmonary tuberculosis (PTB) in humans definitely spreads through respiratory droplets[6]. Pulmonary tuberculosis (PTB) starts at the alveolar level to capture Mycobacterium tuberculosis into the respiratory tract[7].

M. tuberculosis bacilli are resistant to anti-tuberculosis therapy (ATT), so PTB may be sensitive or resistant to drugs[8]. Drug resistance poses a serious threat to tuberculosis control and prevention programs in poor and developed countries[9]. Multidrug-resistant tuberculosis (MDR-PTB) is caused by a strain of Mycobacterium tuberculosis, at least not responding to rifampicin and isoniazid.[8,10]

Rifampicin is the most effective first-line drug (FLD) used in the intensive and sustained phases[11]. In fresh cases where anti-TB drugs have never been taken, primary drug resistance is now referred to as drug resistance[10].

Secondary drug resistance is not congenital, but is obtained due to ineffective treatment of tuberculosis. In the previously treated cases, it is currently called drug resistance.[12]

Risk factors associated with drug-resistant tuberculosis (DR-TB) are the past history of PTB and its treatment (default and relapse), contact with PTB patients, and patients whose treatment has failed[13].

Isolation of MTB and detection of rifampicin resistance are key components of tuberculosis management[14]. Although amplification techniques are currently fascinating methods of practice, however LJ culture is the gold standard for the diagnosis of *M.tuberculosis* in various low and middle income countries since many years[15]

The study was designed to detect primary and

secondary rifampicin résistance in pulmonary tuberculosis suspects by Lowenstein Jensen media.

MATERIALS AND METHODS:

This cross-sectional study, was carried out in the Department of Microbiology, BMSI, JPMC Karachi. It comprised of sputum Samples from pulmonary tuberculosis suspects of adult age on the clinico- radiological and hematological grounds, was conducted during October 2015 to September 2016. Outdoor patients new and those having past history of anti-tuberculosis treatment were selected from Department of Chest Medicine (Ward-12) while patients on anti-tuberculosis treatment were excluded.

An ethical approval was obtained from the ethical committee of BMSI, JPMC . Informed consent was taken from patients included in the study.

A total of 220 samples were collected and sample size calculated by Open Epi software version 3.03 in reference of the study by Iram et al.[16] Two day protocol for collection of sputa was adopted. All sputum samples were analyzed by LJ culture and Gene Xpert RIF/assay was used as a positive and negative control. All confirmed positive (culture plus Gene Xpert positive) samples were processed for DST by proportion method

RESULTS:

The study involved analysis of 220 sputum samples from pulmonary tuberculosis suspects. Out of 220 samples there were 5(2.27%), 167(75.90) and 48(21.81) culture contaminants, culture positive and negative respectively. Male and female were found around 125 (56.81%), 95 (43.19%).

Out of 167 LJ culture positive patients male and female were 96 (57.48%) and 71(42.51%) respectively. Among 167, 121 (72.45%) being 21-40 years old and mean age was 34.996 ((±11.44) years.

Married and unmarried were found at 100(59.88%) 67(40.11%) respectively. The literacy rate was 63(37.72%) while illiterate were 104 (62.27%). Nonsmokers 97 (58.08%) exceeded than smokers 70 (41.91%). History of TB treatment and contact with MDR-TB cases were found at 35(20.95%) and 45(26.94%). Out of 167 patients, 35 were found RIF resistant. The primary RIF resistance was lower 8 (4.79) than secondary resistance 27 (16.16). Among the secondary RIF resistance 18(10.77%) and 9 (5.38%) were defaulters and relapse cases respectively. The smoking is considered a major risk factor for TB relapse and in this study was found in 24 (68.57%) RIF- resistant cases with strong statistical significance (p 0.021).

TABLE 1. DISTRIBUTION OF LJ CULTURE RESULTS OF PTB SUSPECTED PATIENTS (n=220)

Gender	Contaminants n (%)	Positive n (%)	Negative n (%)	Total n (%)
Male	3(1.363)	96(43.63)	26(11.81)	125 (56.81)
Female	2(0.90)	71(32.27	22(10.0)	95 (43.19)
Total	5(2.27)	167(75.90)	48(21.81)	220 (100)

TABLE 2. COMPARATIVE RESULTS OF LJ CULTURE AND GENE Xpert MTB/RIF ASSAY(n=220)

Method	MTB + ve	MTB -ve	Total	Sensitivity (%)	Specificity (%)
LJ culture	167	48	215	98.23	96.00
Gene Xpert assay	169	51	220	98.255	96.226

TABLE 3. DRUG SENSITIVITY TESTING BY LJ CULTURE (n=167)

Gender	RIF sensitive n(%)	RIF resistant n(%)	Total n(%)
Male	76 (45.50)	20 (11.97)	96 (57.48)
Female	56 (33.53)	15 (8.98)	71 (42.51)
Total	132 (79.04)	35 (20.95)	167 (100)

TABLE 4: DISTRIBUTION OF AGE OF PTB PATIENTS WITH AND WITHOUT RIFAMPICIN RESISTANCE (n=167)

Variables (years)	Patients without RIF resistance 132 (78.10)	Patients with RIF Resistance 35 (21.90)	Mean(±SD) Years	p-value
≥16-20	7 (5.30)	2 (5.71)	34.996 (±11.44)	0.251
21-40	101(76.51)	20 (57.14)		
41-50	13 (9.9)	6 (17.14)		
51-60	6 (4.5)	5 (14.28)		
61-80	5 (3.78)	2 (5.71)		

TABLE 5. COMPARISON OF VARIABLES BETWEEN PTB PATIENTS WITH AND WITHOUT RIFAMPICIN RESISTANCE (n=167)			
Variables	**Patients without RIF resistance 132 (79.04%)**	**Patient with RIF resistance 35 (20.95%)**	**p-value**
Male	76 (45.50)	20 (57.14)	0.380
Married	80 (64.39)	20 (40.54)	0.015
Illiterate	78 (59.09)	26 (75.67)	0.063
Smoker	46 (33.33)	24 (68.57)	0.019
Prior H/O TB treatment			
Yes	8 (6.0)	27 (78.37)	0.000
No	124 (94.0)	8 (21.63)	
Contact with MDR-TB case			
Yes	37 (28.03)	8 (21.62)	0.471
No	95 (71.93)	27 (77.80)	

TABLE 6. FREQUENCY OF PRIMARY AND SECONDARY RIFAMPICIN RESISTANCE (n=167)				
Type of resistance	**Rifampicin Sensitive 132 (79.04)**	**Rifampicin Resistant 35 (20.95)**	**Total 167(100)**	**p-value**
Primary resistance	159 (95.20)	8 (4.79)	167 (100)	0.000
Secondary resistance	140 (83.83)	27 (16.16)	167 (100)	

TABLE 7. FREQUENCY OF DEFAULTERS AND RELAPSE CASES IN POSITIVE PTB PATIENTS (n=167)		
Type of a case	No. of patients	Percentage (%)
Defaulters	18	10.77
Relapse cases	9	5.38
Total	27	16.15

DISCUSSION:

In various studies, rifampicin resistance in pulmonary tuberculosis has been tested at different levels of scope. The present study focused on identification and detection of pulmonary tuberculosis patients with rifampicin resistance by conventional LJ culture method. . Out of 220 samples there were 5(2.27%), 167(75.90) and 48(21.81) culture contaminants, culture positive and negative respectively. This study showed male predominance 125 (56.81) over female 95 (43.19). This was consistent with a report by India.[17]

In this study LJ culture proportion method detected 35 (20.95) RIF resistant cases. This was in agreement with the study by Nazir et al[18]. (2009) from Lahore, Pakistan. Our study showed higher rifampicin resistance among male, 57.14% (20/35) in comparison to female 42.85% (15/35), the male predominance was supported by another study conducted by Gangly et al.[19] However when compared with or without resistance to rifampicin, the difference was statistically insignificant (p=0.380). Our study is supported in this regard by the Yazar et al.[20]The male-to-female ratio (6:1) among rifampicin resistant cases was reflected in another study. This problem may be due to lifestyle factors for tuberculosis such as smoking and intake of alcohol.[19]

Male preponderance for DR-TB is not a widely recognized phenomenon, with several reports showing no gender gap in the incidence of drug resistance.[20] Most patients with rifampicin resistance were aged 21-40 years 20 (57.14%) followed by 41-50 years 6 (17.14) %), which was consistent with a study conducted by Kiran et al.,[13].

Khurram et al.,[21] considered the smoking as a significant risk factor for TB relapse. In our study, smoking was found in 24 (68.57 %) RIF resistant cases with good statistical significance (p 0.019), but Kiran et al[13] showed slightly lower frequency (66%) when compared to our study.

A significant risk factor associated with rifampicin-resistant tuberculosis (p<0.000) is prior tuberculosis treatment this is favoured by Li et al.[22], who concluded that patients with

retreated pulmonary TB are six times more likely to have rifampicin resistant tuberculosis (RR-TB) than newly diagnosed patients with TB.

Our analysis showed negligible values for illiteracy. This was incompatible with a thesis in India.[23]The history of near interaction with MDR-TB cases was 21.62 percent in our analysis, and Mulu.[24] found an almost comparable result.

In this study, primary and secondary resistance to rifampicin was 4.79% (8/167) and 16.16% (29/167) respectively, and these results were correlated with the Adane et al. [25] study. In India and China, the primary resistance to rifampicin was 2.8% and 5.7%, respectively[26].

The outcome of this study showed that secondary resistance to rifampicin was almost four times higher than primary resistance, a result confirmed by previous research[27]. Secondary resistance to rifampicin included 10.77% (18/167) and 5.38% (9/167) consecutively as defaulters and relapsed patients. However, Goswami[28] findings showed higher levels of relapsed cases (44%) compared to our results.

CONCLUSION:

A rapid increase in resistance to rifampicin is a significant threat to TB control programs in Pakistan and other developing countries. In our study primary rifampicin resistance (4.79%) was found to be much lower than secondary RIF resistance(16.16%) in the urban population of Karachi. Our study found that rifampicin resistance was much lower than secondary RIF resistance ((16.16)). Defaulters had more RIF resistance than relapsed patients (10.77 VS 5.38%). Thus, we concluded that TB monitoring systems should be improved to avoid ATT discontinuity, thereby reducing defaulters and cases of relapses.

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Conflict of interest: We declare that there is no dispute of interests regarding the publication of this paper.

Study limitations: Improper and insufficient information regarding MDR Contact and retreatment given by patients miscalculated case reporting and relatively small size.

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