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Rapid Detection of Mutations in rpoB Gene of Rifampicin Resistant *Mycobacterium tuberculosis* Strains by Line Probe Assay in GGG Hospital, Jamnagar, Gujarat, India

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A B S T R A C T

Multidrug resistant (MDR) tuberculosis, resistance to at least rifampicin (RMP) and isoniazid (INH), is an increasing problem both in the developed as well as in the developing countries. The early diagnosis of tuberculosis and the rapid detection of resistance to the major anti-tubercular drugs is therefore, of utmost importance for the effective control of the resurgent epidemic. This study was carried out to detect *rpo B* gene mutations in rifampicin resistant Mycobacterium tuberculosis isolated from MDR suspected sputum samples by line prob assay method. Total 3,346 MDR suspected (Criteria A,B,C) sputum samples were received in TB Culture -DST laboratory, Microbiology department, Guru Govind Singh Hospital, Jamnagar during study period for 6 months from January 2015 to may 2015 and proceeded for direct smear examination, DNA extraction, Amplification and Hybridization and detection of *rpo B* gene mutations and rifampicin resistance by line probe assay method. Out of 3,346 samples, 2210 sputum samples were showing direct smear positive for acid fast bacilli suggestive of Mycobacterium tuberculosis. Out of 2210 direct smear positive samples, total 141 sputum samples were rifampicin resistant *M .tuberculosis* and studied for presence of *rpo B* gene mutations by line probe assay method were most common wild type 8 were 77(54.6%) absent and mutant type 3 were 104(74.0%) present. The present study has shown detection of *rpoB* gene mutations in rifampicin resistance *M.tuberculosis* is helpful to epidemiological purpose to prevent primary treatment failure,to study success rate and detection rate of various geographical area.

Introduction

Tuberculosis is a worldwide public health problem despite the highly effective drugs and vaccine is available making tuberculosis a preventable and curable disease (Joveria Qais Farooqi *et al.*, 2012). Tuberculosis is the second-most common cause of death from infectious disease after those due to HIV/ AIDS (Dolin *et al.*, 2010).

A person with fully susceptible tuberculosis may develop secondary resistance during therapy because of inadequate treatment, not taking the prescribed regimen appropriately or using low-quality medications (O'Brien *et al*., 1994).

Drug-resistant tuberculosis is a serious public health issue in many developing countries, as its treatment is longer and requires more expensive drugs.

Conventional methods for myco-bacteriological culture and drug susceptibility testing are slow and cumbersome, requiring sequential procedures for isolation of mycobacterium from clinical specimens, identification of Mycobacterium tuberculosis complex, and in vitro testing of strains susceptibility to anti-tuberculosis drugs. During this time patients may be inappropriately treated, drug resistant strains may continue to spread, and amplification of resistance may occur. Novel technologies for rapid detection of anti-tuberculosis drug resistance have therefore become a priority in tuberculosis research and development, and molecular line probe assays focused on rapid detection of rifampicin resistance are most advanced (Raviglione *et al*., 2006).

Materials and Methods

Study duration & sample size

Total 3,346 MDR suspected (Criteria A,B,C) sputum samples were studied over a period of 5 months from January 2015 to May 2015 in the TB culture and DST laboratory, department of Microbiology, Guru Govind singh Hospital, Jamnagar

There were total 3,346 MDR suspected(criteria A,B,C) 2 sputum samples, one spot supervised and one early morning collected in sterile screw cap wide mouth falcon tube and transported from various centres received in TB Culture - DST laboratory, Microbiology department, Guru Govind Singh Hospital, Jamnagar during study period.

The sputum samples were smeared for direct microscopy and following the gradings of smears, sputum samples were further proceeded for sputum decontamination, DNA extraction followed by Amplification and Hybridization and *rpo B* gene mutations were detected in refampicin resistant *Mycobacterial tuberculosis* by line probe assay method.

Inclusion and Exclusion criteria

Detection of *rpo B* gene mutation in refampicin resistant mycobacterium tuberculosis isolated from sputum samples by line prob assay method were included and other than sputum samples and sensitivity pattern of refampicin, isoniazid and mutation of isoniazid will be excluded.

Ethical clearance

It was a retrospective analysis of samples collected for routine diagnosis, so ethical consideration was not necessary.

Results and Discussion

Out of 3,346 samples, 2210 sputum samples were showing direct smear positive for acid fast bacilli suggestive of *Mycobacterium tuberculosis* were proceeded for line prob assay method following sputum decontamination and DNA extraction.

Out of 2210 direct smear positive samples, total 141 sputum samples were rifampicin resistant *M .tuberculosis* and studied for presence of *rpo B* gene mutations by line probe assay method as shown in Table 1.

Table -2 shows sex wise distribution of *rpo B* gene mutations in rifampicin resistant *M.tuberculosis* 95(67.00%) were from male patients and 47(33.0%) were from female patients.

Table -3 shows age wise distribution of *rpo B gene* mutations in rifampicin resistant *M.tuberculosis*, 10-20 year were 06 (4.2%), 21-30 year were 57(40.4%) maximum resistant, 31-40 year were 37(26.0%),41-50 year were 24(17%), 51-60 year were 15(11.0%), 61-70 year were 02(1.4%).

Table 4 shows direct smear positive grading wise distribution of *rpo B* gene mutations in rifampicin resistant *M . tuberculosis* were 36(26.0%) grade 1, 46(33%) were grade 2, 40 (28.0%) were grade +3 and 19(13.0%) were scanty.

Table- 5 shows *rpo B* gene mutations in Rifampicin resistant *M.tuberculosis* with wild type 8 were 77(54.6%) absent and 64(45.4%) present, 3 were 18(12.8%) absent and 123(87.2%) present, 4 were 15(11.0%) absent and 126(89.0%) present, 7 were 10 (7.0%) absent and 131(93%) present, 5 were 03(2.0%) absent and 138(98%) present,2 were 02(1.4%) absent and 139(98.6%) present and 1 and 6 were 00(0%) absent and 141(100%) present.

Table 7 shows *rpoB* gene mutation in Rifampicin resistant *M.tuberculosis* with mutant type absent and present were mutant type 3 were 104(74.0%) present and 37(26%) absent, mutant type 2-A were 08(6.0%) present and 133(94.0%) absent, mutant type 2-A were 4(3.0%) present and 137(97%) absent, mutant type 1 were 9(6.4%) present and 132(93.6%) absent.

In present study total direct smear positive pulmonary tuberculosis samples were 2210 out of 3,346 and proceeded for line probe assay.

Out of 2210 direct smear positive samples, total multidrug resistance tuberculosis were 332(9.92%) . Out of it 141(4.21%) were rifampicin mono resistance compared with the study of Nilima Hirani *et al* showed that a total LPA was done on 2506 out of 4264 sputum samples, were 1420(33.3%) MDR, 97(2.27%) were rifampicin mono resistance .In present study as compared to Nilima Hirani *et al*., total MDR were less and total rifampicin resistance were more as shown table 7 (Nilima Hirani *et al*., 2014).

In present study, showed that *rpoB* gene mutation in rifampicin resistance caused by *M.tuberculosis* were more common in male 95(67%) as compared to female 46(33%), compared witth Neeraj raizada *et al* 230(72%) male and 90(28%) female, showed that *rpoB* gene mutations in rifampicin resistant *M.tuberculosis* were more common in male as compared to female,that is similar to present study. The reason may be due to more outdoor activity of male as compared to female as shown in table 8 (Neeraj raizada *et al*., 2014).

In present study,the age-wise prevalence of *rpoB* gene mutations in rifampicin resistant *M. tuberculosis* from sputum samples showed that aged group 21-30years 57 (40.4%),while 31-40 years 37(26.0%),41-50 years 24(17.0%) and 51-60 years 15 (11.0%) ,compared with study of Shittu O Rasaki *et al* were age group 21-30 and 31 - 40 were more affected similar to present study as shown in table 9 (Shittu Rasaki *et al*., 2014).

In present study, the direct smear positive grading-wise prevalence of *rpoB* gene mutation in rifampicin resistant *M. tuberculosis* were grade +2 showed 46(33%),while grade +3 were 40(28%), grade 1 were 36(26%), compared to study of Raj N yadav and Neeraj raizada were

similar to study of Raj n yadav. In study of Neeraj raizada study all grades were more compared to present study and most common was grade +1 as shown in table 10 (Raj yadav *et al*., and Neeraj raizada *et al*., 2014).

Table.1

Total samples	Total Smear positive	Total LPA	Total MDR	Rifampicin resistance
3,346	2210(66.04%)	2210(66.04%)	332(9.92%)	141(4.21%)

Table.2 Sex wise distribution

Sex	Total No.	Percentage
Male	95	67.0%
Female	46	33.0%
Total	141	100%

Table.3 Age wise distribution

Age (years)	Total No.			Percentage
	Male	Female	Total	
10-20	2	04	06	4.2%
21-30	34	23	57	40.4%
31-40	28	09	37	26.0%
41-50	16	08	24	17.0%
51-60	13	02	15	11.0%
61-70	02	00	02	1.4%
Total	95	46	141	100%

Table.4 Direct smear positive grading wise distribution of rpo B gene mutations in rifampicin resistant *M. tuberculosis*

Direct smear grading	Total	Percentage
+ 1	36	26.0%
+2	46	33.%
+3	40	28.0%
Scanty	19	13.0%
Total	141	100%

Table.5 rpoB gene mutations (Wild type) in Rifampicin resistant *M.tuberculosis*

Wild type	Total (Absent)	Percentage (Absent)	Total (Present)	Percentage (Present)
1	00	0%	141	100%
2	02	1.4%	139	98.6%
3	**18**	**12.8%**	123	87.2%
4	**15**	**11.0%**	126	89.0%
5	03	2.0%	138	98.0%
6	00	0%	141	100%
7	**10**	**7.0%**	131	93.0%
8	**77**	**54.6%**	**64**	**45.4%**

Table.6 rpo B gene mutation (Mutant type) in Rifampicin resistant *M.tuberculosis*

Mutant	Total (Present)	Percentage	Total (Absent)	Percentage
1	09	6.4%	132	93.6%
2-A	04	3.0%	137	97.0%
2-B	08	6.0%	133	94.0%
3	**104**	**74.0%**	**37**	**26.0%**

Table.7 Comparition of total samples, total LPA done, total MDR, rifampicin resistance and isoniazide resistance

Study	Total samples	Total LPA	Total MDR	Rifampicin resisntance
Present study	3346	2210(66.04%)	332(9.92%)%)	141(4.21%)
Nilima Hirani	4264	2506(58.77%)	1420(33.3%)	97(2.27%)

Table.8 Comarition of Sex-wise distribution

	Male	Female
Present study	95(67%)	46(33%)
Neeraj raizada	230(72%)	90(28%)

Table.9 Comparition of age-wise distribution

Age range	Present study	ShittuO Rasaki
21-30	57(40.4%)	38(27.1%)
31-40	37(26.0%)	47(33.6%)
41-50	24(17.0%)	27(19.3%)
51-60	15(11.0%)	08(5.7%)

Table.10 Comparition of direct smear positive grading-wise prevalence of rpoB gene mutations

Grade	Present study	Neeraj raizada
+1	36(26%)	110(34.0%)
+2	46(33%)	76(24.0%)
+3	40(28%)	98(31.0%)
Scanty	19(13%)	36(11.0%)

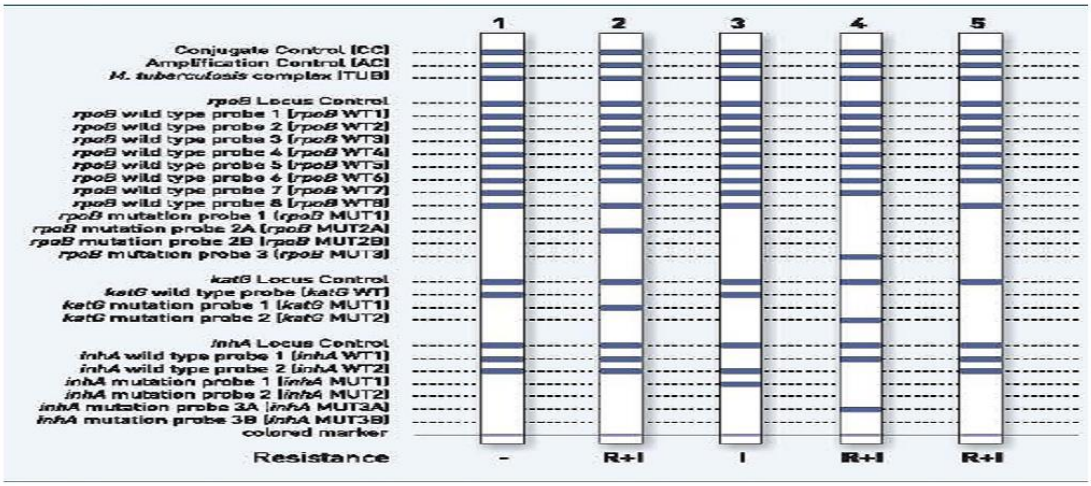
Table.11 Comparition of absence of wild type band in rpoB gene mutation in rifampicin resistant *M. tuberculosis* With different studies

Wild type	Present study	Joveria Qais Farooqi	Raj n yadav	Neeraj Raizada	P.kumar
8	**77(54.6%)**	**36(67.9%)**	2(40%)	12(36%)	**62(63.3%)**
3	18(12.8%)	6(11.3%)	5(100%)	28(85%)	2(2%)
4	15(11%)	6(11.3%)	5(100%)	31(94%)	3(3.1%)
7	10(7.0%)	3(5.6%)	4(80%)	3(97%)	2(2%)

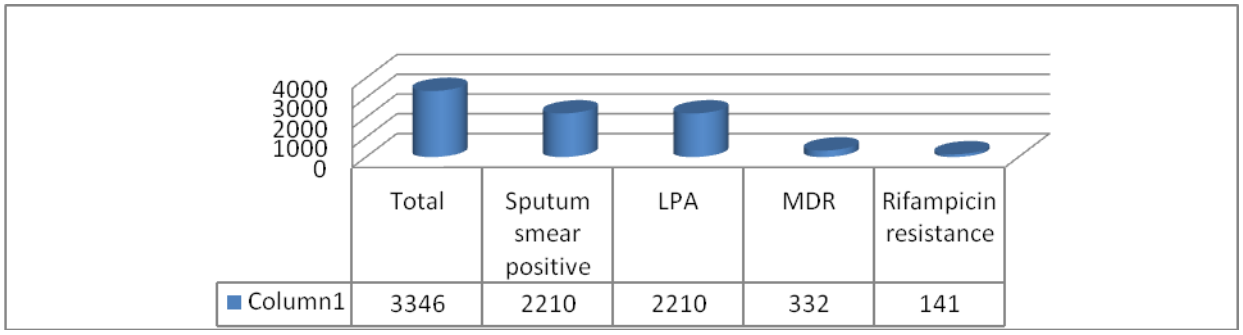
Table.12 Comparition of presence of Mutant tyape band in rpoB gene mutations in rifampicin resistant *M. tuberculosis* with different studies

Mutant Type	Present study	Joveria Qais Farooqi	Raj n yadav	Neeraj Raizada	P.kumar
3	**104(74%)**	**33(62.3%)**	**2(40%)**	**15(46%)**	**39(39.8%)**
1	9(6.4 %)	3(5.7%)	00(0%)	4(12%)	2(2.0%)
2-B	8(6.0%)	00(0%)	00(0%)	00(0%)S	1(1%)
2-A	4(3.0)	2(3.8%)	00(0%)	2(6.0%)	5(5.1%)

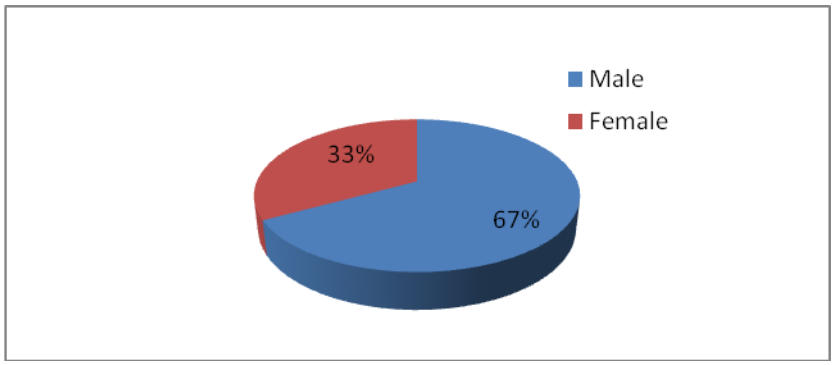
Fig.1



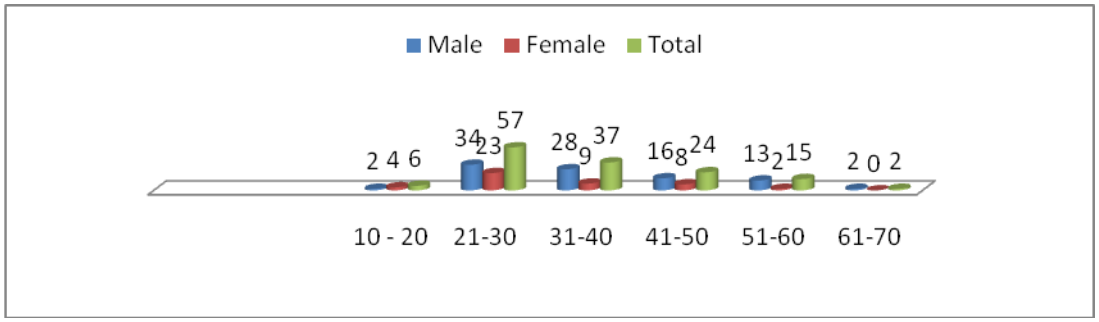
Graph.1



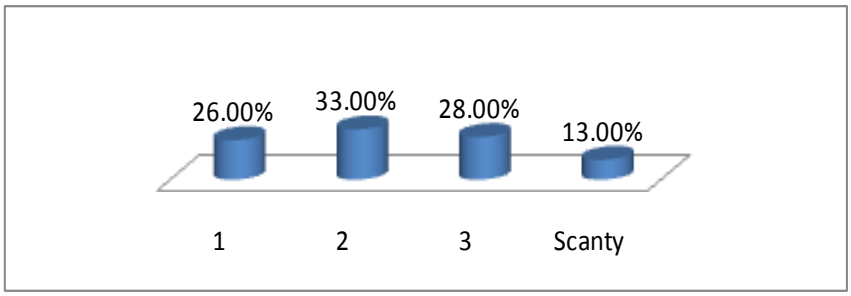
Graph.2 Sex-wise distribution



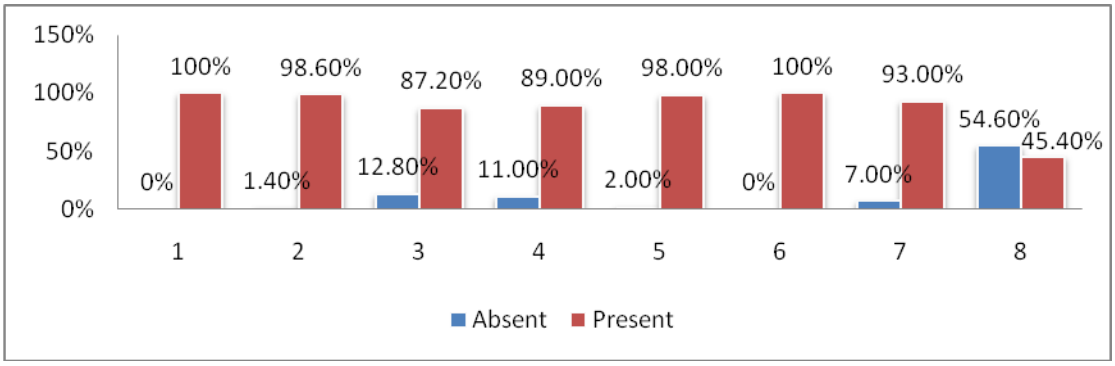
Graph.3 Age-wise distribution



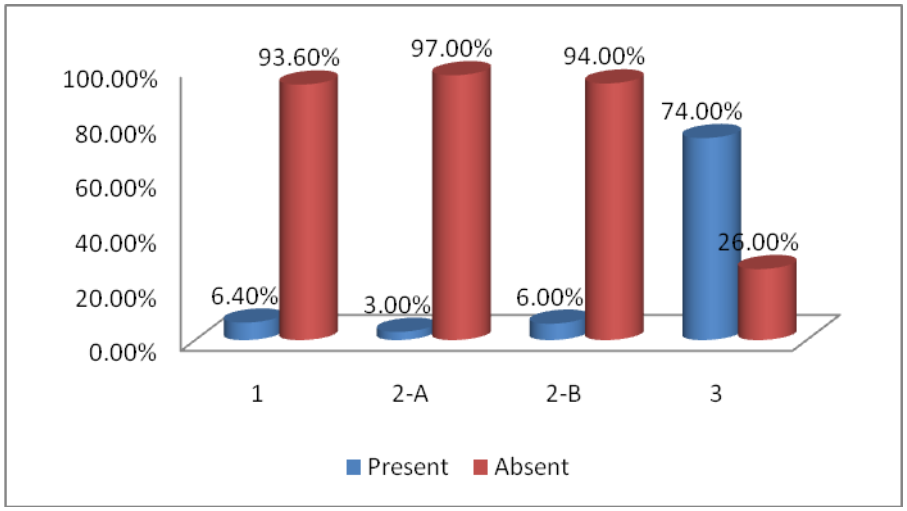
Graph.4 Direct smear positive grading-wise distribution



Graph.5 rpob gene mutation(wild type) in Rifampicin resistant *M.tuberculosis*



Graph.6 rpoB gene mutations (mutant type) in Rifampicin resistant *M.tuberculosis*



In present study, *rpoB* gene mutations in rifampicin resistant *M. tuberculosis* from sputum samples showed that most common wild type absent in *rpo B* gene mutations, wild type 8 were 77(54.6%) while wild type 3 were 18(12.8%) , wild type 4 were 15(11%) and wild type7 were 10(7.0%), were wild type 8 absent was most common compared to study of Joveria Qais Farooqi *et al.*, 36(67.9%), and kumar *et al.*, 62(63.3%) were similar to present study. Were as in study of Raj n yadav 2(40%) and in Neeraj Raizada study was 12(36%).The prevalence of wild type 8 was more in present study as compared to other studies as shown in table 11(Joveria Qais Farooqi *et al.*, 2012; Raj yadav *et al.*, 2013 *and* kumar *et al.*, 2015).

In present study, *rpoB* gene mutations in rifampicin resistant *M. tuberculosis* from sputum samples showed that most common mutant type present in *rpo B* gene mutations, most common Mutant type 3 were 104(74%),while Mutant type 1 were 9(6.4%), Mutant type 2-B 8(6.0%) and mutant type 2-A 4(3%) ,compared to study of Joveria Qais Farooqi 33(62.3%), Raj n yadav 2(40%), Neeraj Raizada 15(46%) and P.nayak 39(39.8%). The prevalence was more in presence study as compared to other studies. In comparison studies most common mutant type was mutant 3,similar to present studyas shown in table 12(Joveria Qais Farooqi *et al.*,2012; Raj yadav *et al.*, 2013; Neeraj raizada *et al.*, 2014; kumar *et al.*, 2015).

In conclusion, the presence study has shown detection of *rpo B* gene mutations in rifampicin resistant *M.tuberculosis* is important factor to know patient's drug responce, treatment failure, relapse rate, reinfection of pulmonary tuberculosis. Early detection of *rpo B* gene mutations in rifampicin resistance is helpful to start MDR TB treatment and to prevent XDR TB. Line probe assay can be helpful to detect *rpoB* gene mutations and to detect which type of mutations are more common in rifampicin resistance. Study is also helpful to epidemiological purpose to prevent primary treatment failure. It is also useful to study the success rate and detection rate of various geographical area. Thus the INNO LiPA Rif TB proved to be a simple, rapid and reliable tool both for the identification of *M. tuberculosis* and for the characterization of the *rpoB* gene mutations responsible for the resistance to rifampicin before the results of the conventional method are available. This assay has also been used to detect the presence of *M. tuberculosis* complex and its resistance to rifampicin directly from clinical samples and needs further evaluation. The LPA test provides an early diagnosis of mono resistance to isoniazid and rifampicin and is highly sensitive and specific for an early diagnosis of MDR-TB. Based on these findings, it is concluded that the LPA test can be useful in early diagnosis of drug resistant TB in high TB burden countries (Meera Sharma *et al*., 2013).

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