Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2022i1.165
*Biol. Clin. Sci. Res. J., Volume, **2022**: 165*
Original Research Article

COMPARISON OF GENEXPERT® PROBE MISSING IN HOTSPOT RRDR OF *RPOB* GENE AMONG PRIMARY AND ACQUIRED DRUG RESISTANT CASES OF PULMONARY TUBERCULOSIS

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(Received, 7th June 2022, Revised 22th December 2022, Published 25th December 2022)

Abstract: *There are differences in probe pattern missing in rifampicin resistance determining region of rpoB gene among primary and acquired resistant TB cases. This study aimed to observe the Xpert® MTB RIF Assay presenting probe missing in Hotspot RRDR of rpoB Gene among primary and acquired drug-resistant cases of pulmonary tuberculosis in a tertiary care setting of Lahore. This comparative cross-sectional work was undertaken at The University of Lahore in cooperation with the Department of Pulmonology and HRI-NIH TB Research Centre KEMU Lahore. Confirmed TB patients being sent for Xpert® MTB RIF Assay were the target population in this study. Based on previous TB treatment history, patients were divided into primary and acquired drug-resistant TB cases. Samples were subjected to Xpert® MTB RIF Assay, and any probes missing, i.e., probes A, B, C, D or E, were observed. Data from a total of 170 cases were finalized for analysis consisting of 84(49.4%) acquired TB cases and 86(50.6%) primary TB cases. A total of 90(52.9%) male and 80(47.1%) females were enrolled. The mean age of patients was 35.45±16.11 years. The proportion of missing probe E (78.2%) remained at the topmost, followed by none of the probes missing at 8.3%, probe A at 5.3%, probe C at 3.5%, probe B at 2.9% and probe D at 1.8%. There are variations in frequencies of probe missing in RDRR regions of rpoB genes among rifampicin-resistant cases of primary and acquired TB. A high frequency of no missing probes among primary resistant was found*.

Keywords: Genotyping, *Mycobacterium tuberculosis*, MTB complex, Epidemiology, Diagnosis

Introduction

Tuberculosis (TB) is a foremost global health concern and is categorized as rampant by World Health Organization (WHO). TB affects the lungs though it can affect every tissue and body organ in the circulatory system, lymphatic system, central nervous system, and dietary system. It can affect almost any part of the body. (Patterson et al., 2017) One-third of the global population is estimated to be infected with MTB and at risk of developing the disease. The estimated global incidence rate of TB remained at 127 cases per 100,000 population, and 1.5 million people died of TB in 2020, including 214000 Human Immunodeficient viruses (HIV) positive cases. (WHO)

Substantial development Goals (SDGs) are set by the WHO to End TB strategy and consist of various milestones and global targets for reducing the incidence of TB and related deaths. According to SDG-3, the global TB epidemic must be ended by 2030, and a reduction in 35% of deaths and 20% rate of incidence was expected by 2020, which remains below half by 2020 and Global reduction in death toll remained to be 14% and incidence as 9%. Pakistan is facing a high incidence of 263/100,000 cases of all types of TB, and the death rate due to TB remained 19.9/at 100,000. (WHO) The considerable influence of TB is posed on the socioeconomic status of Pakistan as the country bears 5.8% of TB alone from the total national disease burden, where the country is ranked as the 6th highest TB cases containing nation. (WHO)

Multi-drug resistant (MDR) TB is the disease caused by the strain of the *Mycobacterium tuberculosis* (MTB) complex, which is resistant to at least isoniazid and rifampicin with or without resistance to any other first-line anti-TB drugs. Basic diagnostic readily available laboratory tests to diagnose TB are observation of acid-fast bacilli (AFB) by smear

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microscopy and MTB culture on Lowenstein Jensen (LJ) medium in clinically suspected cases. (Bayot et al.) Early diagnosis with definite microbiological evidence is an essential effective treatment and good patient care, thus controlling the further spread of TB. On the other hand, due to a delayed definitive diagnosis, clinical and therapeutic decisions are to be established before laboratory diagnosis for the sake of patients and at-risk populations.(Kesarwani et al., 2004)

Induction of GeneXpert® in diagnostic facilities has revolutionized the diagnosis of TB and drug-resistant TB, overwhelming various limitations of culture and smear techniques. Various targets of DNA sequences are used to confer rifampicin resistance, and around ≥95% of mutations in rifampicin-resistant strains are encoded in 81 bp rifampicin resistance determining region (RRDR) of the *rpo*B gene. Mutations occurring in the RRDR region are the most probably predictive of rifampicin. Additionally, the core region of the *rpo*B gene is fringed by MTB-specific DNA sequences. Therefore, it became possible to use a single amplicon by PCR to test MTB and rifampicin resistance simultaneously. (Lawn and Nicol, 2011)

Five variable types of acid hybridization probes labeled Probe A, B, C, D and E are used in one multiplex reaction. Each probe is paired to a specific target sequence within the 81 bp core region of the *rpo*B gene in rifampicin susceptible MTB and is labeled with a different tinted fluorophore suppression of any probes during amplification labels the organism resistant to rifampicin. The frequency of probe missing varies in different regions; other differences in the frequency of probe missing in primary and acquired cases are unknown in Pakistan. Therefore, the present study was undertaken to observe the Xpert® MTB RIF Assay presenting probe missing in Hotspot RRDR of *rpoB* Gene among primary and acquired drug-resistant cases of pulmonary tuberculosis in a tertiary care setting of Lahore.

Methodology

This comparative cross-sectional work was conducted in the Department of Molecular Biology & Biotechnology at The University of Lahore in cooperation with the Department of Pulmonology and HRI-NIH TB Research Centre KEMU Lahore. The study was conducted from July 2020 to March 2022. The target population in this study was confirmed TB patients being sent for Xpert® MTB RIF Assay to check the status of rifampicin resistance.

Based on the previous history of TB treatment, patients were divided into two groups. Patients taking a prior TB treatment for at least four weeks were labelled acquired TB cases, while patients with no prior history of TB treatment were labeled as primary patients. A minimum sample size of 84 patients in each group (168) was statistically calculated. A convenient sampling technique was used, and the number of rifampicin-resistant cases for both groups was completed prospectively.

After taking informed consent, patients were asked to submit 2-4 ml guided sputum samples. Each sample was mixed vigorously with a lysing reagent provided with the kit and incubated for 15 minutes at room temperature. Two ml of each specimen were shifted in separate pre-labeled test cartridges and loaded to GeneXpert® through software. After two hours, results were noted from the software for any probes missing, i.e., probe A, B, C, D or E. Data was entered and analyzed in the statistical package of social sciences (SPSS) software.

Results

Data from 170 cases were finalized for analysis consisting of 84(49.4%) acquired TB cases and 86(50.6%) primary TB cases. 90(52.9%) male patients remained higher compared to 80(47.1%) females. The mean age of patients was 35.45±16.11 years. Distribution of other characteristics, including marital status, education and smear results according to the primary and acquired drug-resistant groups, are presented in Table 1

Weight loss (98.2%) has remained the most predominant symptom, followed by fever (97.6%), cough (96.5%) and fatigue (93.5%) cases. A comparative view of symptoms among both groups is presented in Figure 1. There was an observed probe missing among primary and acquired TB patients, variable frequency of each probe was observed, and the Chi-square test was applied; however, the difference remained insignificant (p-value >0.05) in most of the cases on individual probe missing cases. A significant difference (p-value <0.05) was observed among cases conferred resistance with none of the missing probes, and a higher number of primary drug-resistant cases presented this finding.

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Table 1: Characteristics of Rifampicin Resistant TB Cases

Characteristics		Group				Total	
		Acquired		Primary			
		n	%	n	%	n	%
Gender	Male	42	46.7	48	53.3	90	52.9
	Female	42	52.5	38	47.5	80	47.1
Marital Status	Married	55	49.1	57	50.9	112	65.9
	Unmarried	29	50.0	29	50.0	58	34.1
Education	Illiterate	32	53.3	28	46.7	60	35.3
	Primary	16	43.2	21	56.8	37	21.8
	Middle	13	48.1	14	51.9	27	15.9
	High	20	50.0	20	50.0	40	23.5
	Higher Above	3	50.0	3	50.0	6	3.5
Smear Result	Scanty	5	55.6	4	44.4	9	5.3
	1+	23	39.0	36	61.0	59	34.7
	2+	33	48.5	35	51.5	68	40.0
	3+	23	67.6	11	32.4	34	20.0

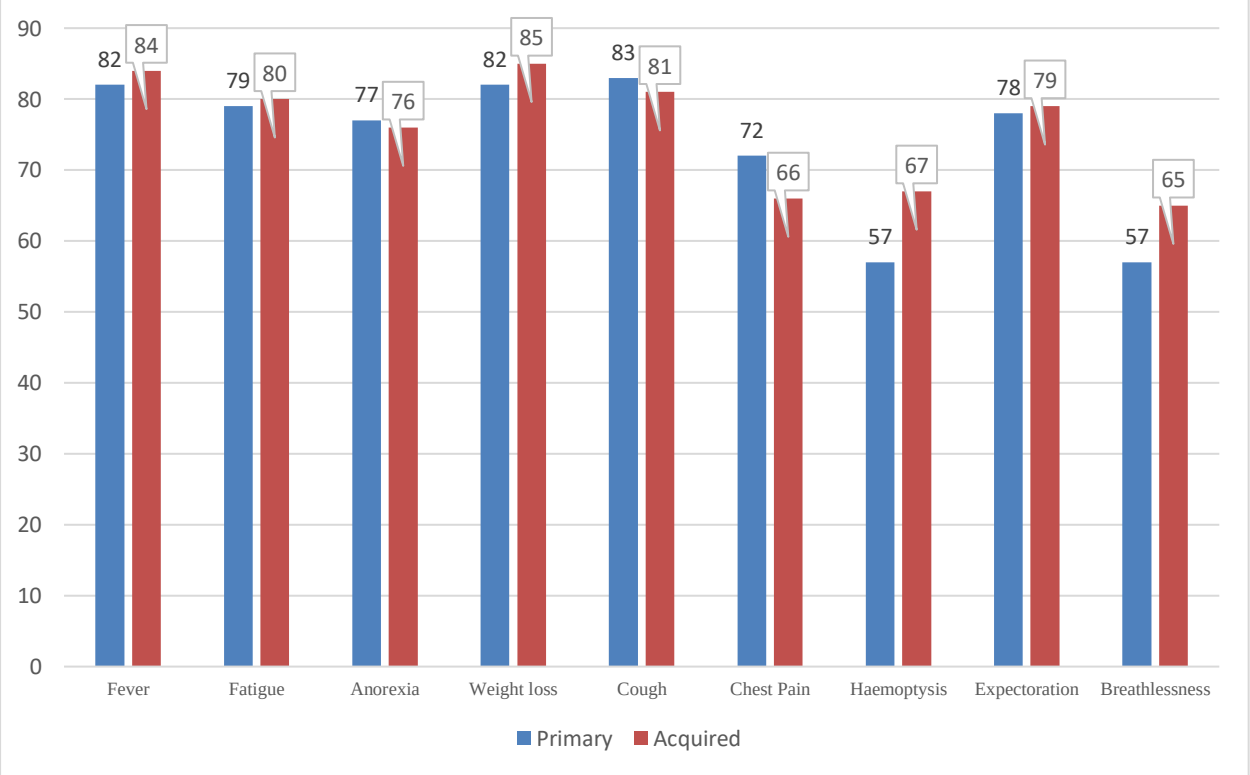

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Figure 1: Comparative view of Symptoms among primary and acquired TB Patients

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Table 2: Comparison of Probe Missing among Both Groups

Probe Missing	Acquired N=84		Primary N=86		Total N=170		p-value
	n	%	n	%	n	%	
None	1	1.2	13	15.1	14	8.3	0.001
A	7	8.4	2	2.3	9	5.3	0.077
B	4	4.7	1	1.2	5	2.9	0.177
C	4	4.7	2	2.3	6	3.5	0.395
D	2	2.4	1	1.2	3	1.8	0.556
E	66	78.6	67	77.9	133	78.2	0.912

Discussion

The prevalence of many strains along diverse virulence and pattern of resistance categorizes the distribution of TB in various geographic areas. (Welekidan et al., 2021) Various host and environmental factors are thought to be the reason for the transmission of various strains of MTB. (Bussi and Gutierrez, 2019) An essential phase of the TB control program must be its capacity to demonstrate the mode and site of transmission for prevention from further spread of infection and active TB by finding recently infected individuals and providing treatment, further observing treatment completion leading to cure. Such practice has aided in finding the transmission connections among individuals and determining instances in which associated cases were infected through distinct strains.(Moreno-Molina et al., 2021)

The presently proportion of missing probe E (78.2%) remained at the topmost, followed by none of the probes missing at 8.3%, probe A at 5.3%, probe C at 3.5%, probe B at 2.9% and probe D at 1.8%. Comparable findings are presented by a study that presented probe E as the most common probe liable to confer rifampicin resistance in amon83.82% of cases, followed by probes D, B and C, respectively. Probe A alone was not conferred any resistance but was shown to be present in the combination of 2 or three missing probes. (Kanade et al., 2019)None of the probes missing in 8.3% of cases predominantly presented in primary rifampicin-resistant cases was reported in the above-cited study. (Kanade et al., 2019)

Results regarding mutation in probe E (81.0%) & probe B (3.0%) were also presented concomitant to the present findings, while differences may be observed in the case of probe D (10%). No mutations in probe A and probe C. Similarly, findings agree with this study, where 6.0% of cases confer mutations in the *rpoB* gene without missing probe type. (Alemu et al., 2020) A more recent study to identify the missing probes in pulmonary and extra-pulmonary TB cases also reported promising results to present findings and revealed 77.41% missing probe E. In contrast, combinations of probes C, D and D, E remained at 3.22% each. (Kumar et al., 2020). A study undertaken in 2016 reported a lower frequency of missing probe E at 64.1%, followed by probe B at 15.2%, probe D at 14.1%, probe C at 3.3%, probe A at 2.2% and no probe at 1.1%.(Rahman et al., 2016) Hence above findings are not comparable with the present study. Other demographic and symptomatology of primary and acquired TB patients are concurrent to the findings already published in literature from the same settings and region. (Kashif Munir et al., 2021)

In conclusion, it is pertinent that there are variations in frequencies of probe missing in RDRR regions of *rpoB* genes among rifampicin-resistant cases of primary and acquired TB. The main feature of this study is an observation of no missing probes among primary resistant cases, which is not focused on yet nor reported definitely. Differences in mutation patterns are observed compared to the studies from other regions.

Funding Source This is not funded work while the facility of GeneXpert® and drug testing was provided by Health Research Institute National Institutes of Health (HRI-NIH) Council TB Research Centre, King Edward Medical University Lahore, Punjab, Pakistan.

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Conflict of Interest: Authors declare that they have no conflict of interest.

Ethics Approval: Ethical approval for this study was obtained from the Institutional Review Board of King Edward Medical University through letter No. 438/RC/KEMU dated 4th July 2020, and it is certified that study was performed following ethical standards as laid down in 1964 Declaration of Helsinki and its later amendments.

Consent to participate: Written consent from each patient was obtained to participate in this study.
Consent for publication: Written consent from each patient was obtained for data publication, keeping in view the confidentiality of individual information.

Author's contribution: All authors have contributed significantly to this study. MKM conceived and designed the project, MKM and SR performed the experimental work, MKM and SS wrote the paper, SS and AH clinically evaluated the cases, SS and AH revised the manuscript, and MKM and SR did the data analysis.

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