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ORIGINAL ARTICLE

PREVALENCE OF RIFAMPICIN RESISTANCE AND PROBES IDENTIFICATION OF 81BP RRDR RPO- β GENE IN PULMONARY TUBERCULOSIS POPULATION OF DISTRICT BANNU, PAKISTAN

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ABSTRACT

Background: Tuberculosis is major public health problem in developing countries due to emergence of drug resistance. The objectives of this study were to detect the prevalence of rifampicin resistance (RR) and probe mutation in 81bp rifampicin resistance determining region (RRDR) of rpo-β gene in pulmonary TB population of District Bannu, Pakistan.

Materials and Methods: This cross-sectional study was conducted in Department of Pathology, Bannu Medical College, Bannu, Pakistan from March 2021-June 2021. Total 1,382 MTB detected pulmonary isolates by Xpert MTB/RIF assay were enrolled from January 2014-December 2018. Presence of RR and presence of probes of mutation, sex, age groups and category of disease were variables. All nominal variables were analyzed by count and percentage.

Results: Prevalence of RR was 75 (5.43%) (95%CI 4.35-6.74) in 1,382 MTB detected pulmonary isolates. It was similar in men 37 (2.68%) & women 38 (2.75%), and in new 37 (2.68%) & previously treated patients 38 (2.75%). It was highest 2.10% for age group 15-29 years. The mutation detected in 81bp rpo-β gene was highest in probe E 42 (3.03%), followed by B 16 (1.15%), D 09 (0.65%), A 04 (0.28%), C 2 (0.14%), and B&D & E&D 01 (0.07%) each.

Conclusion: Our study showed the prevalence of RR 5.43% in MTB detected pulmonary isolates. It was similar in men & women, and in new & previously treated patients. It was highest for age group 15-29 years. The most common probe was E, followed by B, D, A, C, B&D and E&D.

KEY WORDS: Mycobacterium Tuberculosis; Rifampicin; RNA; Gene; Pulmonary Tuberculosis; Multi Drug Resistant Tuberculosis; Confidence Interval; RNA Probes; Rifampicin; Polymerase Chain Reaction.

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1. INTRODUCTION

1.1 Background: Tuberculosis is a major public health problem in the developing world. It is estimated that 10 million people in the world get TB infection annually with 1.7 million deaths.[1] Pakistan ranks 5th in the estimated global TB burden list.[2,3] Multi-drug-resistant tuberculosis (MDR-TB) is a major public health issue, particularly in developing countries.[4,5] World Health Organization (WHO) has reported 600,000 individuals with rifampicin-resistant tuberculosis (RR-TB) and 490,000 of these RR cases develop MDR-TB. Among those RR-TB cases, 47% of these patients belong to India, China, Russian Federation and Pakistan.[6,7] According to WHO 2018 report, Pakistan is one of the 27 high MDR-TB burden countries with an MDR-TB rate of 4.2 % in new (category-I) cases and 16% among the previously treated (category-II) TB cases.[8,9] The increase in MDR-TB cases is correlated to treatment

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failure & relapse, which exhibits extra difficulties in TB control.[10] RR has a precise epidemiological variety and is a valuable marker for MDR-TB strains since 90% or above of those strains resistant to rifampicin (RIF) are also resistant to isoniazid which are said to be MDR-TB. RIF is the key first-line anti-TB drug that works by inhibiting the synthesis of ribonucleic acid (RNA) directed by the deoxyribonucleic acid (DNA) of mycobacterium tuberculosis (MTB) proteins by binding to the β subunit of the bacterial DNA-dependent RNA polymerase enzyme protein (rpo-β).[11] Mutations in the rpo-β gene are reported as 95 to 97% of RR-MTB strains throughout the world which is usually situated in a region at the 507-533 amino acid residues (81bp) within the rpo-β genetic feature known as RR determining region (RRDR).[5,12]

Laboratory diagnosis of tuberculosis (TB) is primarily done by a simple method of smear microscopy using Zeil Nelson (ZN) staining method.[13] Other methods of diagnosis are also available like fluorescent microscopy (FM), culturing of bacteria on artificial media and an advanced molecular technique called Xpert MTB/RIF assay.[14] PCR-based methods developed over the last decades have signifcantly improved and accelerated diagnosis of drug-resistant tuberculosis (DR-TB); however such methods require a specfific molecular diagnostic laboratory setup. The need for simpler PCR systems has been solved with the real-time PCR-based system Xpert MTB/RIF assay. It can simultaneously detect MTB and RR in less than two hours. Since 2013, the GeneXpert test is being recommended by WHO as an initial diagnostic test for patients with suspected pulmonary TB, including new, retreatment cases, suspected/ contact multidrug-resistant (MDR) TB and HIV-infected patients with chest complications because of its high sensitivity and specificity associated with a tremendously short turnaround time. Detection of rpo-β gene mutations is considered a surrogate marker for MDR-TB detection and can be used as a tool in MDR-TB diagnostics. The patterns and frequency of mutations in the RRDR region of the rpo-β gene in the MTB clinical isolates vary significantly according to the geographical location. Limited data is available regarding the pattern of rpo-β mutation in the MDR-TB patients in Pakistan.[15-17]

1.2 Research Objectives (RO)

RO 1-4: To determine the prevalence of rifampicin resistance (RR) and its distribution by sex, age groups and type of disease in pulmonary TB population of District Bannu, Pakistan.

RO 5-8: To determine the prevalence of different probes in 81bp rifampicin resistance determining region (RRDR) of rpo-β gene and its distribution by sex, age groups and type of disease in pulmonary TB population of District Bannu, Pakistan.

1.3 Significance

No such study has been conducted before in our population. It will help the clinicians in best treatment strategies for eradicating TB and will serve as baseline data for future research.

2. MATERIALS AND METHODS

2.1 Study design, settings, duration & sampling: This cross-sectional study was conducted in the Department of Pathology, Bannu Medical College, Bannu, Pakistan from March 2021 to June 2021. District TB Center, Bannu received 20,134 pulmonary TB suspect cases from January 2014 to December 2018. Out of these 20,134 cases, 1,965 were processed for GeneXpert, including 1,581 (80.46%) category-I and 384 (19.54%) category-II patients. Out of these 1,965 cases, 1382 (70.33%) cases were mycobacterium tuberculosis (MTB) positive. These 1382 MTB positive cases was our sample.

2.2 Diagnosis of the patients: Those patients complaining of cough for more than two weeks, moderate pyrexia in the evening time, chest pain, sputum containing blood, low body mass/ height ratio was advised early morning sputum specimen for the sputum microscopy and Xpert MTB/RIF assay to detect MTB level (semi-quantitatively) and RR.

2.3 Sample selection criteria for Xpert MTB/ RIF assay: The criteria of sample selection for processing on Xpert MTB/RIF assay in District Bannu from 2014 to 2018 was such that all the category-II (once completed anti-tuberculosis treatment), new patients having contact with MDR-TB patients, new smear-positive follow up cases on 2nd/ 5th month of treatment and fresh clinically suspected of having MDR-TB cases were processed.

2.4 Xpert MTB/RIF assay: The specimens for Xpert MTB/RIF assay were processed using the standard N-Acetyl-L-cysteine–NaOH technique. N-Acetyl-L-cysteine–NaOH was added to the specimen in a closed sterile container in a 2:1 ratio for de-contamination and liquefaction of the mucus sample.[18] Using a fresh transfer pipette, 2 ml of the prepared specimen was transferred to an Xpert MTB/Rif assay cartridge. The cartridge was then loaded into the specified module of the GeneXpert machine (Cepheid, Sunnyvale, CA, USA) as per manufacturer standard protocol. The interpretation is software-based. The result was recorded as MTB detected (high, medium, low, and very low)/ MTB not detected and rifampicin-resistance (RR) detected/ not detected/ in determinant. The in determinant cases were counted as RR cases. The report also contained the detail about the probes of the rpo-β gene which are involved in the causation of the resistance to rifampicin.

2.5 Data collection & analysis plan: The data was collected from TB/ GeneXpert register. Presence of RR and presence of probes of mutation were our two research variables, while sex, age groups and category of disease were our demographic/ grouping variables/ factors. All variable were on nominal scale. All these variables were analyzed by count and percentage with 95%CI using Wilson score interval by an online statistical calculator "Statistics Kingdom".[19] The probes of mutation were determined using a software built-in laptop system connected with a GeneXpert machine. The data was analyzed using IBM SPSS Statistics Version 25.0, released 2017 (Armonk, NY: IBM Corp.).

3. RESULTS

3.1 Prevalence of RR and its distribution by sex, age groups and category: The prevalence of RR was 75 (5.43%) (95%CI 4.35-6.74) in 1,382 MTB detected cases.

Out of 1,382 MTB detected cases, the prevalence of RR was similar in men 37 (2.68%) & women 38 (2.75%) and in new patients 37 (2.68%) and in previously treated patients 38 (2.75%). It was highest in age group 15-29 years 29 (2.10%) than other age groups. (Table 3.1)

The mean age of the sample was 36.40±17.4 (13-80, range 67) (95%CI, 32.39-40.41) years.

3.2 Prevalence of different probes and their distribution by sex, age groups and category: The prevalence of mutant probes detected was 75 (5.43%) (95%CI 4.35-6.74) in 1,382 MTB detected cases. The prevalence of different mutant probes in 1,382 MTB detected cases was highest in probe E 42 (3.04%), followed by B 16 (1.16%), D 09 (0.65%), A 04 (0.29%), C 02 (0.15%), B&D 01 (0.07%) and E&D 01 (0.07%).

Out of 1,382 MTB detected cases, the prevalence of mutant probes was similar in men 37 (2.68%) & women 38 (2.75%) and in new patients 37 (2.68%) and in previously treated patients 38 (2.75%). It was highest in age group 15-29 years 29 (2.10%) than other age groups. (Table 3.2)

Table 3.1: Distribution of RR by sex, age groups and category in MTB detected pulmonary TB population of District Bannu, Pakistan (n=75/1,382)

Variables	Attributes	Rifampicin resistance		Sample statistics		95%CI	
		In determinant	Detected	Count	Percentage	Lower	Upper
Sex	Men	1	36	37	37*100/1382=2.68	1.94	3.66
	Women	2	36	38	38*100/1382=2.75	2.00	3.75
Age groups (years)	0-14	0	3	3	3*100/1382=0.22	0.07	0.63
	15-29	1	28	29	29*100/1382=2.10	1.46	2.99
	30-44	1	19	20	20*100/1382=1.45	0.93	2.22
	45+	1	22	23	23*100/1382=1.66	1.11	2.48
Category	New patients	2	35	37	37*100/1382=2.68	1.94	3.66
	Previously treated patients	1	37	38	38*100/1382=2.75	2.00	3.75
RR cases		3	72	75	75*100/1382=5.43	04.35	06.74
No RR cases				1307	1307*100/1382=94.57	93.25	95.64
Total MTB cases				1382	100%		

Table 3.2: Distribution of different probes in 81bp rpo-β gene by sex, age groups and category in MTB detected pulmonary TB population of District Bannu, Pakistan (n=75/1,382)

Variables A		Probe types							Sample statistics	95%CI		
		B	C	D	E	B&D	E&D	Count	%age	Lower	Upper	
Sex	Men	2	6	1	2	24	1	1	37	2.68	1.94	3.66
	Women	2	10	1	7	18	0	0	38	2.75	2.00	3.75
Age groups (years)	0-14	0	1	0	0	0	0	0	03	0.22	0.07	0.63
	15-29	1	5	0	2	22	1	0	29	2.10	1.46	2.99
	30-44	0	4	0	4	12	0	0	20	1.45	0.93	2.22
	45+	3	6	2	3	8	0	1	23	1.66	1.11	2.48
Category	New patients	2	9	1	3	21	1	0	37	2.68	1.94	3.66
	Previously treated patients	2	7	1	6	21	0	1	38	2.75	2.00	3.75
Probe types Count & %age		4 (0.29)	16 (1.16)	2 (0.15)	9 (0.65)	42 (3.04)	1 (0.07)	1 (0.07)	75 (5.43)	5.43	04.35	06.74

rpo-β: RNA polymerase beta, bp: base pair

4. DISCUSSION

4.1 Prevalence of RR and its distribution by sex, age groups and category: Rifampicin, a most potent first-line anti-tuberculosis drug has significance in the diagnosis of MDR-TB in today's world by using GeneXpert technology which detects the main mutation in β subunit of the rpo-β gene, which causes resistance to the most potent first line anti-TB drug rifampicin (RIF). It is known that 90% of the strains resistant to RIF are also resistant to isoniazid.[20] Xpert MTB/RIF assay is a new advanced real-time PCR based test used over the past decade. Due to its high sensitivity, it has great significance.

In our population, the prevalence of RRD was 5.43% (95% CI 4.35-6.74) i.e. 75 out of 1,382 pulmonary MTB detected cases. Similar prevalence of 6% was reported by Qazi, et al.[21] in 2014 from Lahore, Pakistan as 63 out of 1,080 isolates.

All other studies given below showed higher prevalence than our population.

Ullah, et al.[22] in 2016 from Peshawar, Pakistan reported 28.97% (408*100/1408), probably due to altered sample selection criteria. He has enrolled 1,408 pulmonary and extra-pulmonary MTB positive patients diagnosed by Xpert MTB/RIF assay. Bakuła et al.[23] in 2019 from Punjab, Pakistan, reported higher rate of 14% in the most populated province of Pakistan. Gautam, et al.[24] from Uttar Pradesh, India in 2018 showed the prevalence of RR as 26.19% (44*100/168=27.69) in 168 MTB pulmonary and extrapulmonary cases on Xpert MTB/RIF assay. Jahan, et al.[25] from Dhaka, Bangladesh reported 20.9% (14 out of 67 pulmonary MTB cases) prevalence of RR on GeneXpert MTB/RIF in year 2014. Arega, et al.[26] in 2019 from Ethiopia reported prevalence of RR as 15.11% and Ikuabe, et al.[27] in 2018 from Nigeria as 14.7%. This higher rate may be due to different sample selection criteria and the larger populations.

In our population of 1,382 MTB cases, the prevalence of RR was similar in men 2.68% (37*100/1382=2.68) and women 2.75% (38*100/1382=2.75).

Contrarily, Ullah, et al.[22] reported higher prevalence of RR in women 17.25% (243*100/1408) than men 11.72% (165*100/1408) in 2016 from Peshawar. Likewise, Gautam, et al.[24] identified 26.19% (44*100/168=27.69) RR cases out 168 MTB pulmonary and extrapulmonary cases. The prevalence of RR was higher in men 22.62% (38*100/168=22.62) than women 3.57% (6*100/168=3.5).

In our population of 1,382 MTB cases, the prevalence of RR was highest 2.10% (29*100/1382) in age group 15-29 years, followed by 1.66% (23*100/1382) in 45+ years, 1.45% (20*100/1382) in 30-44 years and 0.22% (3*100/1382) in 0-14 years.

Ullah, et al.[22] reported highest prevalence of RR 11.15% (157*100/1408) in age group 15-24 years, followed by 10.86% (153*100/1408) in 25-44 years, 4.90% (69*100/1408) in 45+ years and 2.06% (29*100/1408) in 0-14 years. Gautam, et al.[24] reported highest prevalence of RR 14.88% (25*100/168) in age group 20-40 years, followed by 7.74%

(13*100/168) in 41-60 years, 2.38% (4*100/168) in 61-80 years and 1.19% (2*100/168) in 0-20 years.

In our population of 1,382 MTB cases, the prevalence of RR was similar in new patients 2.68% (37*100/1382=2.68) and previously treated patients 2.75% (38*100/1382=2.75).

In contrast, Ullah, et al.[22] reported higher prevalence of RR in previously treated patients 25.99% (366*100/1408=2.68) than new patients 2.98% (42*100/1408=2.98). Likewise, Gautam, et al.[24] reported higher prevalence of RR in previously treated patients 23.21% (39*100/168=23.21) than new patients 2.98% (5*100/168=2.98).

4.2 Prevalence of different probes and their distribution by sex, age groups and category: In our study, 75 (5.43%) mutations were detected by Xpert MTB/RIF assay in RRDR 81bp rpo-β gene. The mutations were highest in probe E 42 (3.04%), followed by B 16 (1.16%), D 09 (0.65%), A 04 (0.29%), C 02 (0.15%), B&D 01 (0.07%) and E&D 01 (0.07%).

Similarly, Ullah et al.[22] in 2016 from Peshawar, Pakistan found highest prevalence in probe E 22.30% (314*100/1408), followed by B 3.12% (44*100/1408), D 2.41% (34*100/1408), C 0.43% (6*100/1408), A 0.36% (5*100/1408), B&D 0.21 (3*100/1408), A&B 0.07 (1*100/1408) and E&D 0.07 (1*100/1408).

Khan, et al.[28] from Punjab, Pakistan reported probe E with highest prevalence of 52% among RR cases as no data of total MTB detected cases was shown in this study. 512 region was detected for the first time in Pakistan in this study. Yue et al.[29] in 2003 reported from China highest prevalence in probe E 41%. No data of total MTB positive cases was available. Mboowa et al.[30] in 2014 also reported that 2.2% (7*100/313) mutations were in probe E in Kampala, Uganda. Similarly, high frequencies of this mutation in probe E were found in isolates from Korea (38%),[31] Japan (33%),[32] Italy (30%),[33] and Greece (19%);[34] in all such studies no data about the MTB detected cases was found.

The difference between the current study and other studies conducted in different parts of the world may be due to geographical disparity, sample size, socio-economic status of different populations and the methodology for selection of presumptive patients for MDR-TB.

5. CONCLUSION

Our study showed the prevalence of RR 5.43% in MTB detected pulmonary isolates. It was similar in men & women, and in new & previously treated patients. It was highest for age group 15-29 years. The most common probe was E, followed by B, D, A, C, B&D and E&D. Future recommendation is to extend the sample selection criteria for Xpert MTB/RIF assay in this population for capturing hidden TB cases.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

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Acquisition, Analysis or Interpretation of Data:	AAK, HUK, ZK, IK, AR, AUR, QNUR, MR, MS
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All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.