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ORIGINAL ARTICLE

Frequency of Fluoroquinolone and Aminoglycoside Resistance Online Probe Assay in Multidrug-Resistant Tuberculosis Patients

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ABSTRACT

Background: The resistance of tuberculosis to anti-tuberculous therapy is a severe public health concern all over the globe. The most common and important cause of resistance to anti-tuberculous therapy is poor patient compliance with or inappropriate therapy dosing.

Objective: In order to evaluate the prevalence of resistance to commonly used 2nd-line anti-tuberculous drugs, an online probe assay was conducted among patients diagnosed with multi-drug-resistant tuberculosis. This study specifically focused on patients who were receiving treatment with fluoroquinolones and aminoglycosides and were seeking care at Lady Reading Hospital, a tertiary care facility located in Peshawar.

Methods: This study was conducted at the Department of Pulmonology ward of Lady Reading Hospital, Peshawar, from Jan 2021 to Jan 2022, six months in which a total of 150 patients were observed to determine the frequency of common 2nd-line standard anti-tuberculous drugs resistance online probe assay in patients with multidrug-resistant tuberculosis. flouroquinolones and . Non-probability sequential sampling was the method used for sampling.

Results: This study, conducted at the Department of Pulmonology ward of Lady Reading Hospital in Peshawar, employed a cross-sectional design. The objective was to assess the prevalence of resistance to standard 2nd-line anti-tuberculous drugs among patients with multidrug-resistant tuberculosis using online probe assays. A total of 150 patients were included in the analysis (n = 15-24). The age distribution of the participants was as follows: 52 patients (34.7%) were between 25-34 years old, 34 patients (22.7%) were between 35-44 years old, and 35 patients (23.3%) were between 45-54 years old (Table No. 1). The mean age was found to be 33.76 years, with a standard deviation of 16.42. In terms of gender, 66 patients (44%) were male and 84 patients (56%) were female (Figure No. 1). Sputum culture conversion at 2 months was observed in 50.7% of the cases, while it was absent in 49.3% (Table No. 3). Additionally, previous tuberculosis treatment was reported by 5 patients (0.7%), while the majority (99.3%) had no history of previous treatment (Table No. 6).

Conclusion: The use of fluoroquinolones standard antia was the A considerable percentage of resistance was discovered in the nation's first attempt to test the extent of 2nd-line medication resistance among MDR-TB isolates. Even though there were 84 females present, it's interesting that none of the isolates fit the requirements for XDR-TB. A crucial indicator of the probable severe pan resistance development of XDR-TB is the use of first-line drugs. While the national TB control plans are put into place, MDR-TB cases must be closely monitored. The short: Regularity, proximity

Keywords: 2nd-line standard anti-tuberculous drugs resistance, multidrug-resistant tuberculosis. Tuberculosis fluoroquinolones and aminoglycosides,

INTRODUCTION

Resistance of tuberculosis to anti-tuberculous therapy is a severe public health concern all over the globe. The most common and important cause of resistance to anti-tuberculous therapy is poor patient compliance with or inappropriate dosing of therapy1. Thus patients who develop multidrug-resistant tuberculosis are usually the ones who have had previous exposure to anti-tuberculous therapy; however, sometimes resistance can also arise in patients who have never previously taken any drugs2. This occurs because of infection with bacilli that are already resistant.Multi-drug resistant tuberculosis (MDR TB) refers to tuberculosis that is resistant to both isoniazid and rifampicin. 3 Multidrug-resistant tuberculosis is most prevalent in developing nations for a variety of reasons, including a lack of professional specialist opinion, inadequate infrastructure, poverty, difficulties getting drugs, lack of vigilance, and many more. Pakistan, which ranks fifth in the world in terms of the number of MDR TB cases, is another emerging nation that significantly contributes to the multidrug-resistant tuberculosis burden4.The short-drug therapy regimen, which has a total length of 9 to 11 months, is a standardized treatment plan for chosen individuals with multidrug-resistant TB.5. [Kanamycin], [moxifloxacin], [prothionamide], [clofazimine], [pyrazinamide], Ethambutol and high-dose isoniazid are given for 4 to 6 months, then 5 months of moxifloxacin, clofazimine, pyrazinamide, and ethambutol are given. (4-6 Km-Mfx-Pto-Cfz-Z-H).High-Dose-E (Mfx-Cfz-Z-E/5.0 Mfx-E) 6. Patients with rifampicin- or multidrug-resistant tuberculosis (MDR/RR TB) strains who are not resistant to fluoroquinolones or 2nd-line injectable drugs may consider this regimen as their first option.6According to a research that was published in the "European Respiratory Journal" in 2013, the prevalence of fluoroquinolone resistance in MDR-TB patients was 6.34 percent, the frequency of aminoglycoside resistance was 16.81 percent, and the frequency of combined fluoroquinolone and aminoglycoside resistance was 6.02 percent.6.one of the eligibility criteria for starting a patient on short drug treatment regimen is the absence of any resistance to 2nd-line drug including(fluoroquinolones and aminoglycosides)7. Therefore, This study is essential to find out the local burden of patients in whom shorter drug treatment regimens can be used and those in whom this regimen cannot be used[7]. This knowledge will help the authorities decide what policies to implement locally to better control drug resistance to tuberculosis.8

MATERIALS AND METHODS

Study Design: Cross-sectional Study

Setting: Pulmonology ward of Lady Reading Hospital, Peshawar

Duration of Study: Minimum of 6 months after approval of synopsis from Jan 2021 to Jan 2022

Sample Size: The sample consisted of at least 136 persons. The (World Health Organization) "Sample Size Determination in Health Studies" software was used to make the determination. The formula for "Estimating a population proportion with specified absolute precision" was applied based on the following assumptions:• Strength of the confidence interval: 95% • Absolute accuracy: 4%

• Anticipated proportion of fluoroquinolone resistance in MDR

tuberculosis patients: 426/6724 = 6.34%7

• Anticipated proportion of aminoglycoside resistance in MDR
tuberculosis patients: 1130/6724 = 16.81%7
• Anticipated proportion of aminoglycoside+fluoroquinolone resistance
in MDR tuberculosis patients: 405/6724 = 6.02%7(The least of the
three percentages and, therefore, the one which was used for calculating sample size).

Sampling Technique: Non-probability consecutive sampling
Sample Selection:

Inclusion Criteria:

• 20 to 70 years old patients of either gender with multidrug-resistant tuberculosis
admitted to the pulmonology ward.
• No previous exposure to 1 or more 2nd-line medicines in the shorter MDR TB
regimen for more than 1 month.

Exclusion Criteria:

• Patients with known resistance to 2nd line drugs.
• Patients with extra-pulmonary tuberculosis will also be excluded from this study.

Data Collection Procedure: The study was conducted after approval from the hospital ethics and research committee.

The patients meeting the inclusion criteria in the pulmonology ward of Lady Reading Hospital,

Peshawar was recruited in the study after taking written informed consent. All of the patients who were recruited for the research were informed of its objectives and its specific requirements. The operational definitions above served as the basis for the diagnosis of multidrug-resistant TB. A thorough medical history was obtained from the patients, including information on their age, gender, the length of their TB, whether they lived in rural or urban areas, their level of education, and any co-existing diseases including diabetes and high blood pressure.. These patients will undergo line probe testing to look for resistance to fluoroquinolones and aminoglycosides, as described earlier in the operational definition.

Based on the test results, the patients were started on the shorter drug treatment regimen if the

The patient also meets the other eligibility criteria recommended by WHO for starting a patient on

Shorter regimen.

Data Analysis Procedure: The data were entered and saved in [SPSS version 23]. [Descriptive statistics] were utilized to examine the information. For categorical factors including gender, residence status (rural/urban), educational level, co-morbidities such hypertension and diabetes, and presence or absence of fluoroquinolone and aminoglycoside resistance, frequencies and percentages were estimated. For the numerical factors, such as age, height duration, weight, and body mass index in connection to TB, mean and standard deviation were computed. Age, gender, educational level, place of residence, co-morbid condition, and BMI were stratified as impact modifiers to investigate their effects on the outcomes. The post-stratification chi-squared test was used for the analysis, and a p-value of 0.05 or less was considered significant. Tables and graphs were used to present all of the data.

RESULTS

In this study, 150 cases were observed to determine the frequency of fluoroquinolone and aminoglycoside resistance. The age ranges of the patients observed were between 18 and 77 years, with an average age of 33.76 (SD 16.42). The Distribution of age-wise Distribution is shown in Table I. Gender-wise distribution among 150 patients was male 66 (44%) and female 84 (56%) (Figure 1). Sputum culture conversion at 2 months was present among 150 (50.7%) and was absent among 149 (49.3%) (Table II). Previous tuberculosis treatment was present among 77 (0.7%) and was absent among 73 (49.3%)(Table III). Fluoroquinolone resistance was present among 45(30%) patients, and aminoglycoside resistance was present among 69(46%) patients. Both fluoroquinolone and aminoglycoside resistance was seen in 25(16.7%)patients. (Table no: IV)

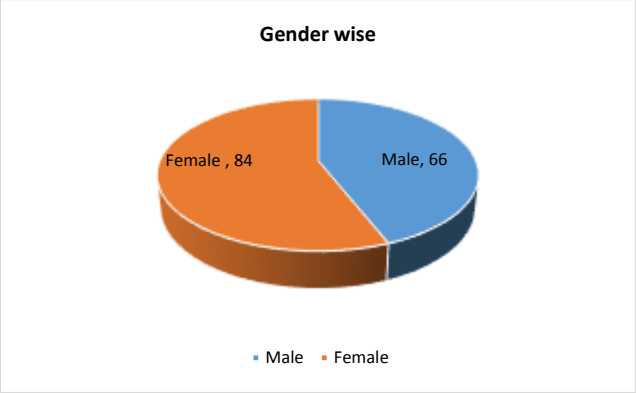

Figure 1: Gender Wise Distribution Sample Size (No 150)

Table 1: Age Wise Distribution among 150 Patients

15-24 Years	52	(34.7%)
25-34 Years	34	(22.7%)
35-44 Years	29	(19.3%)
45-54Years	35	(23.3%)

Table 2: Sputum Culture Conversion at 2 Months and Previous Tuberculosis Treatment

Present among	(50.7%)
Absent among	(49.3%)
Previous Tuberculosis Treatment	
Present among 5	(0.7%)
Absent among	(49.3%)

Table 3: Sputum Culture Conversion at 2 Months

Frequency of fluoroquinolone resistance among patients:	<10%
Frequency of aminoglycoside resistance among patients:	<6%
Previous Tuberculosis Treatment	
Frequency of fluoroquinolone resistance among patients	<5%
Frequency of aminoglycoside resistance among patients:	<3%

Table 4: Province/ Region (No 150)

Province/ Region				
	[Frequency]	[Percent]	[Percent]	[Cumulative Percent]
FATA	[14]	[9.3]	[9.3]	[9.3]
KPK	135	90.0	90.0	99.3
Ningarhar	1	.7	.7	100.0
Total	150	100.0	100.0	

Table 5: Year-Wise Treatment (No 150)

YEAR				
	[Frequency]	[Percent]	[Valid Percent]	[Cumulative Percent]
2017	[02]	[1.3]	[1.3]	[1.3]
2018	104	69.3	69.3	70.7
2019	44	29.3	29.3	100.0
Total	150	100.0	100.0	

Table 6: Result Of The Diagnostic Culture (No 150)

Result of the Diagnostic culture				
	[Frequency]	[Percent]	[Valid Percent]	[Cumulative Percent]
	[45]	[30.0]	[30.0]	[30.0]
H	7	4.7	4.7	34.7
HR	28	18.7	18.7	53.3
HRE	5	3.3	3.3	56.7
HREZ	18	12.0	12.0	68.7
HREZS	3	2.0	2.0	70.7
HRS	4	2.7	2.7	73.3
HRZ	21	14.0	14.0	87.3
HRZS	5	3.3	3.3	90.7
HZ	2	1.3	1.3	92.0
Other (please insert a comment and specify)	10	6.7	6.7	98.7
R	1	.7	.7	99.3
REZ	1	.7	.7	100.0
Total	150	100.0	100.0	

Table 7: Treatment Strategy (No 150)

Treatment Strategy				
	Frequency	Percent	Valid Percent	Cumulative Percent
LTR(Shifted in 1m)	43	28.7	28.7	28.7
STR	107	71.3	71.3	100.0
Total	150	100.0	100.0	

DISCUSSION

The study is the first of its type to be conducted in Saudi Arabia and analyzes 2nd-line medication susceptibility of MDR-TB isolates with prospective molecular characterisation on a nationwide collection9. The MDR-TB isolates that were examined had a number of instances of 2nd-line drug resistance, but no XDR-TB phenotypes were discovered. 100% concordance was found in the tests for genotypic and phenotypic susceptibility.10. The fact that there aren't many mutations other than the typical ones covered by line probe assays in the nation and that there aren't enough instances of [2nd-line] resistance (less than eight cases per medication) to draw any firm conclusions may be the cause of the enormous concordance.In the nation's diagnostic labs, testing for [genotypic]or[phenotypic resistance] to [2nd-line] anti-TB medications is not often done11. The two target genes, gyrA and rrs, which are primarily responsible for conferring resistance to fluoroquinolone and aminoglycoside drugs, were therefore the subject of this investigation, which provided some insight into the potential 2nd-line drug resistance pattern.53% of the patients in the present research of prior therapy, whereas fourty seven percent were fresh cases. Except for combination (resistance) to INH, RIF, and EMB, all drug-resistance patterns were shown to be predominate (> 50%) among the isolates with a prior treatment history13. This is consistent with a prior study that showed individuals who had previously received TB treatment were more likely to acquire or develop MDR-TB as a result of the introduction of drug-resistant strains throughout the nation14. However, it has also recently been reported15 that Saudi natives and immigrants are more likely to get drug-resistant types of bacteria. Additionally, a recent study by Varghese and colleagues found a unique pattern of TB transmission, especially in newly diagnosed immigrant patients. This demonstrates the potential for a distant infection to reactivate, followed by a reinfection with a drug-resistant strain, such (MDR-TB)16. The most discoveries support the notion that the only likely sources of drug-resistant tuberculosis in Saudi Arabia are acquired resistance and recent transmissions. In comparison to other large-scale investigations, the drug resistance pattern revealed a greater incidence of pan-resistant isolates (50.6%), which is very high and suggests the establishment of XDR-TB17. Codon 531 of the rpoB dominated the mutation study for RIF resistance, with codon 526 coming in second place (13.2%)17. This data clearly supports the findings of one of our most recent national surveys and other papers, which identified a high codon 531 mutation rate main contributor to (RIF resistance in Saudi Arabia). In contrast, INH resistance was mostly caused by mutations at codon 315 of the katG gene and position 15 of the inhA promoter region, which is consistent with our earlier results and other research from across the world16. 12% of the MDR-TB isolates under study were resistant to fluoroquinolones; the mutation was mostly found in the gyrA gene at codons 90 and 9417. The two most prevalent variants found (D94G and A90V). a conferring mutation to the rrs gene at position (1401) was discovered in all isolates with aminoglycoside resistance. Other nations have similarly documented the frequency of these alterations among MDR-TB and XDR-TB isolates18. Along with examining the isolates' phylogenetic profiles and associated alterations, we also looked at the demographic background of the patients. 53.8% of the 13 patients who had drug resistance to the second line were immigrants. With around 09.03 million (31.08%) immigrants among its (29.2) million citizens, Saudi Arabia has a distinctive demographic structure.38 According to recent data, immigrants reported more cases of TB each year than Saudi citizens (11.5 cases/100,000 populations), at a rate of 26.7 cases per 100,000 people. 19. A greater prevalence was recorded among the non-Saudi population (5%) compared to the local population (3%) although the total MDR-TB burden among newly diagnosed patients in the nation remains at just 4%.20. Therefore, it is not surprising that immigrants have a greater overall prevalence of treatment resistance, especially to 2nd-line medications. The majority of non-Saudi patients were listed as freshly diagnosed TB cases with no prior history of treatment. This interesting discovery is largely supported by the fact that distant TB infection reactivation is common among immigrants, and the majority come from Asian and African countries with high TB burdens. Numerous nations that receive immigrants have provided proof of this fact21.The isolates' phylogenetic tree revealed that PGG-I (Indo-Oceanic, East-African Indian) lineages predominated (61.5%). This supports earlier research that found that MDR isolates, notably XDR-TB, have greater representation rates of the Beijing, Delhi/CAS, and EAI clades. This research has several restrictions: Due to the small size of the study group, phylogenetic analysis on transmission dynamics could not be performed, and the elevated low resistance of moxifloxacin was not tested with higher concentrations, genome sequencing techniques were not considered to confirm the detected mutations or to find unknown or new mutations, and22.

Limitations: The present study was limited to secondary care multidrug resistance in tuberculosis patients and was only conducted at a single tertiary care facility. Hence the findings may not be highly generalizable. Moreover, the sample size was relatively small, reducing the results' power. Furthermore, the data was collected by self-reported questionnaires regarding past medical history, which may lead to recall bias.

CONCLUSION

a significant amount of resistance was found in the nation's first screening of the extent of (2nd-line) medication (resistance among MDR-TB isolates). It's interesting that none of the isolates met the criteria for XDR-TB. However, the considerable level of first-line medication pan resistance points to the impending development of XDR-TB. The implementation of TB control programs must be closely monitored on a national level for MDR-TB patients.

Future Finding: Further research should be understand better the trends of resistance to the common 2nd-line drugs used to treat multidrug-resistant tuberculosis. Such research should include not only the frequency of resistance but also demographic and other socio-economic factors that may play a role in the development of resistance. Investigating the potential genetic mutations responsible for the resistance to anti-TB drugs is also necessary. Additionally, new technologies should be used to monitor the fluctuations in the resistance patterns to implement timely intervention strategies.

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