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## Frequency of drug-resistant *Mycobacterium Tuberculosis* in Chiniot, Pakistan

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### ABSTRACT

**Introduction:** Tuberculosis is one of the major causes of morbidity and mortality among developing countries, and conditions are getting worse with the emergence of antibiotic-resistant strains of *Mycobacterium tuberculosis* (MTB). Pakistan is trying to deal with TB but a substantial number of cases are still recorded every year.

**Materials and methods:** A cross-sectional study was designed and sputum samples from the suspected individuals were tested for acid-fast bacilli (AFB) and samples were further evaluated for the frequency of rifampicin-resistant MTB by Cepheid Xpert® MTB/Rif assay. The diagnosis of patients for TB Frequency was made based on clinical signs and symptoms with the lab results.

**Results:** The present study enrolled 6110 suspected individuals, out of which 900 (14.7%) were diagnosed with TB of which 511(8.3%) were males and 389(6.3%) were females. The highest (18.4%) frequency of TB cases was found in ages 45-54 (years) and substantially high (16.5%) TB cases were observed in ages 15-24 (years). Results indicated that 18 (0.3%) patients have been established with rifampicin-resistant TB (RR-TB) and among those cases, 13(0.21%) and 5(0.08%) were males and females respectively.

**Conclusion:** The current study concluded that a substantial number of rifampicin-resistant TB (RR-TB) cases were existing more in males than females from different areas of Chiniot, Pakistan and Xpert® MTB/Rif assay was observed to be an accurate and rapid tool to diagnose MTB.

**Keywords:** Tuberculosis, frequency, antibiotics, rifampicin resistance

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### INTRODUCTION

Tuberculosis (TB) is the 9th leading cause of death throughout the globe and about 10 million new

cases of TB are notified every year. In 2016, the world witnessed 1.3 million deaths of people affected by HIV-negative TB and the loss of 374,000 HIV-

positive people (1). TB is an airborne infection caused by *Mycobacterium tuberculosis* (MTB). Poor socioeconomic situation and overcrowding in developing countries are a major cause of MTB aerosol spread and it is more prevalent in males than females (2, 3).

*Mycobacterium* can infect any site in the body (lymph nodes, pleura, GIT, bones, CNS, and Urogenital track) with up to 85% of pulmonary TB (4) and up to 15% cases were of extrapulmonary TB and sites can (5). MTB-contaminated aerosols get access to alveolar macrophages and tubercle bacilli multiply, survive within macrophages, and spread via bloodstream or lymphatics to any part of the body. About 2 to 8 weeks later a cellular immune response is elicited and cellular immunity encapsulates or kills bacteria. When encapsulated, bacteria cannot further spread and cause infection, this condition is known as latent TB, in latent TB body is immunologically active but there are no signs and symptoms of active TB. At any point in life if the person becomes immune compromised these enclosed bacteria start multiplying which leads to Active TB (6, 7).

Patients presented with a cough lasting for two weeks or more along with fever of no proper etiology and weight loss are suspected of TB. The diagnosis is made using chest X-ray, Ziehl-Neelsen staining of sputum smear, and examining it under light microscopy, culturing on LJ media, histological biopsies, and by molecular assays like Xpert® MTB/Rif (8). The diagnosis of TB in the lab is mostly made through ZN staining of a sputum sample, but it has the lowest sensitivity among all the diagnostic tools. Culture is considered a gold standard, but this method takes a long time to generate results. The best diagnostic tool for respiratory samples is Xpert® MTB/Rif assay, it's highly specific, and reports result in just 2 hours, along with Rifampicin resistance status (9). The first-line drugs are rifampicin and isoniazid (10). Globally antibiotic resistance is becoming a crucial problem. *Mycobacterial* resistance to both of the first-line drugs is called multidrug resistance TB (MDR-TB) and resistance to fluoroquinolone and at least one of the second-line injectable drugs like amikacin, Kanamycin, and Capreomycin is called extensively drug-resistant TB (XDR-TB) (11, 12).

Drug resistance in MTB is caused by a mutation in a gene that code for the target site of the drug. It has been evident that MTB encodes for many drug multidrug transporters that depend upon proton motive force (PMF) or ATP, these transporters efflux the drug out of the cell, and evading bacterium from the lethal antimicrobial activity of drugs (13). Rifampicin targets the RNA polymerase, mutation in the gene that codes

for the beta subunit of RNA polymerase leads to the development of resistant bacterial strains against the drug (14). Pakistan ranks fifth among high burden TB countries and ranks the fourth among high burden drug-resistant TB countries (15). The Frequency of bacteriologically positive TB in > 15-year-old population is 389/100,000 (16).

The objective of the current study is to observe the frequency of MTB and the incidence of Rifampicin-Resistance TB in the suspected population of district Chiniot, Pakistan.

## METHODOLOGY

### Study design

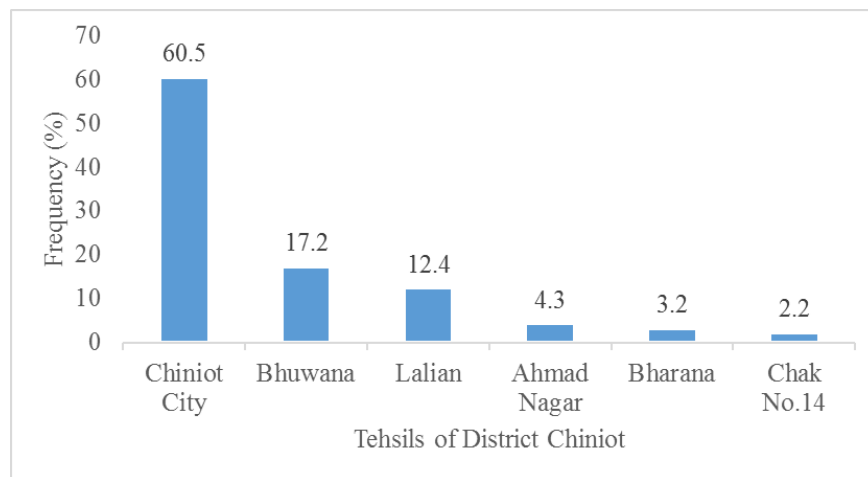
An observational study was conducted which based on the data of tuberculosis suspected individuals that visited eleven different Tehsil Headquarter and District Hospitals in different areas of Chiniot Punjab, Pakistan from April 2019 to September 2019. Individuals having signs and symptoms (cough, weight loss, and fever, blood in sputum, and spots in chest X-ray) of TB that visited the hospital first time and relapse TB cases were included in the study.

### Lab analysis

Two sputum samples, on alternate days, were collected from the TB suspected individuals that didn't have a history of the previous infection with TB, and one sample was collected from relapse TB cases. Samples were analyzed for AFB screening (0.5% carbon fuchsin, 25% Sulphuric acid, 0.3% methylene blue or malachite green) and TB final diagnosis was made on MTB Gene Xpert (Cepheid) analysis in respective labs of eleven different Tehsil Headquarter and District Hospitals in different areas of Chiniot Punjab (17, 18). The study was approved by the ethical committee of the University Institute of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Lahore. Study subjects were diagnosed based on AFB screening and clinical signs & symptoms. AFB smear-positive cases were characterized as 'bacteriological MTB' and other AFB smear-negative were categorized 'clinical MTB' with the detection of the spot on chest X-ray. The data was precisely tabulated in Microsoft Excel to limit the risk of error and the frequencies (%) were calculated.

## RESULTS

In the present study, 900 (14.7%) of total 6110 suspected individuals were found positive for the frequency of MTB, and 545 out of a total 900 (60.5%) positive TB cases were prevailing in Chiniot city, 155 (17%) were in Bhuwana, 112 (12.4%) in Lalian, 39 (4.3%) in Ahmed Nagar, 29 (3.2%) in Barana, and 20 (2.2%) in Chak No. 14 (Figure 1).



**Figure 1:** Frequency of Tuberculosis in different tehsils (Towns) of Chiniot district

Among 900 TB diagnosed patients 511(56%) were males and 389(43%) were females. Study did not find significant relationship ( $p$ -value=0.523) between gender and MTB infection. The highest number of infected TB male patients was 103 (11.5%) from the 45-54years age group. The age group 15-24 indicated the highest incidence of females 70 (8%) in table 1.

Eight hundred and fifteen (90.56%) from a total of 900 patients were newly diagnosed with pulmonary TB and 83 (9.2%) cases were diagnosed with extrapulmonary TB in table 2.

**Table 1:** Age and gender-wise distribution of all TB diagnosed patients

Age (Years)	Gender		Total No. (%)
	Male No. (%)	Female No. (%)	
0-4	1 (0.1)	7(0.7)	8 (0.8)
5-14	19 (2.1)	60(6.6)	79 (8.7)
15-24	79 (8.7)	70(7.7)	149 (16.5)
25-34	59 (6.5)	53(5.8)	112 (12.4)
35-44	75 (8.3)	44((4.8)	119(13.)
45-54	103 (11.5)	63(7)	166 (18.4)
55-64	78(8.6)	48(5.3)	126 (1)
>65	97 (10.7)	44(4.8)	141 (15.6)
<b>Total</b>	<b>511 (56.7)</b>	<b>389(43.23)</b>	<b>900(100)</b>

**Table 2. Distribution of confirmed TB Cases based on diagnostic procedures**

Type of tuberculosis (TB)	Confirmation on diagnostic procedures	Cases of TB		Total
		New No. (%)	Relapse No. (%)	
Pulmonary	Clinically	366 (40.7)	21 (2.4)	387(43)
	Bacteriologically	371(41.2)	59(6.5)	430(47.8)
Extrapulmonary	Clinically	54(6)	5(0.5)	59(6.6)
	Bacteriological	24(2.7)	-	24(2.7)
<b>Total</b>		<b>815(90.5)</b>	<b>85(9.4)</b>	<b>900(100)</b>

Among the total 900 TB cases, 430 (47.78%) were bacteriologically confirmed with 371 (41.12%) newly diagnosed cases, and 59(6.5%) relapse TB infection. Clinically diagnosed individuals were 387(43%) where 366 (40.67%) were newly diagnosed for TB and 21 (2.34%) patients had a relapse infection as in table 2. A total of 24 (2.67%) bacteriologically diagnosed cases were confirmed for MTB and 59 (6.5%) cases were diagnosed with extrapulmonary on clinical ground.

It has been established from the results that 18 (2%) out of 900 MTB patients had an infection of Rifampicin-resistant MTB strains. Thirteen (72%) out of these 18 patients were males and 5(27.78%) were females with the highest percentage (28%) of 5 patients from 15-24 and 25-34 years of age. The highest frequency 2 (11%) of RR-MTB was observed in 25-34 years of females and males were frequent 4 (22%) in age group (15-24 years) for resistance MTB infection with a non-significant relationship of TB frequency and gender (table 3).

**Table 3: Age and gender-wise distribution of Rifampicin resistance in TB**

Age (Years)	Gender		Total No. (%)
	Male No. (%)	Female No. (%)	
5-14	-	1(5.6)	1(5.6)
15-24	4(22.3)	1(5.6)	5(27.8)
25-34	3(16.7)	2(11.2)	5(27.8)
35-44	-	-	-
45-54	2(11.2)	-	2(11.2)
55-64	1(5.6)	1(5.6)	2(11.2)
>65	3(16.7)	-	3(16.7)
<b>Total</b>	<b>13(72.3)</b>	<b>5(27.8)</b>	<b>18(100)</b>

## DISCUSSION

About 10.2 million cases of new and relapse TB were recorded worldwide in 2015, which reduced to 9.02 million in 2016. A drop in TB cases has been noticed in developed countries because health organizations are being equipped with modern tools to detect TB in the early stage with great accuracy, treating the disease in a juvenile state, and regular follow-ups of patients are being implemented (19, 20).

The rate of new TB cases is very low among developed countries. In the United States, the incidence rate of TB is 0.002% as compared to the current study from different areas of Punjab, Pakistan in which 6110 suspected individuals were enrolled and the Frequency recorded was 14% (21). A substantial number of TB cases were recorded in developing countries. A study from 2006-2012 in India reported 0.17%-0.52% of TB burden (22). A high Frequency of TB in Madhya

Pradesh, India was reported in 2010 of 0.423% (23). Not all areas of India are overwhelmed by TB there is a low Frequency recorded in Faridabad district Haryana and a north Indian district of 0.101% and 0.025% respectively in contrast to India's national estimate. In Haryana specifically, the low Frequency is because of the successful execution of TB control measures in the area as stated by Ashutosh, N.A. et al (24, 25). Surveillance data from Dhaka's urban slum, Bangladesh reports the incident rate of TB, 0.253%, which is quite high as compared to developed countries (26).

WHO, in 2000 implemented directed observed treatment and short courses (DOTS), in Pakistan. Adopting DOTS remarkably increased the productivity of health care centers in Pakistan as a result, 85% of positive patients were cured successfully (27). The National TB control program in 2011 attained a TB detection rate of 64% and established an incident rate of 0.23%, but it seems that there is a lag in the record of an unknown number of patients regarding private sectors (28). Pakistan is also overwhelmed by TB, the incident figures for bacteriologically and smear-positive TB is 0.398%, and 0.27%. Another finding of the national TB survey was that 61% of TB patients were positive on sputum smear, as compared to the current study in which 43.89% of smears were positive of total TB patients. The mortality rate was recorded at 0.023% in Pakistan (16).

The incidence rate of TB is higher among males than females globally, male is 1.8 times more probable to TB infection than females (29). In the present study, the incidence rate of TB was more among males than females, 57% and 43% respectively which are relatable to a global report. Similar results from Balochistan, Pakistan are observed among males 57% and females 43%. (30). An unusual spectrum of results is observed in Lower Dir Valley, KPK, Pakistan, in which the frequency of females was slightly higher than males, 49.5%, and 50.4% respectively (31).

Extrapulmonary tuberculosis (EPTB) represents a minority of TB cases. Global TB report states that EPTB makes up 15% of the total TB cases (32). A study from East Sudan informs the incidence rate of 22% of EPTB, as compared to the current study which reports 9.2% (33). The reason for this high Frequency lies in the ignorance of people to the consequences of the diseases, a study conducted in Lahore, Pakistan, reports that 2/3 of patients fail to follow treatment after they are diagnosed with TB (34). These untreated patients then become the culprits who spread infection, a single person infected with TB can infect 10-15 persons annually (35). Failure to receive

treatment is not the only case, medical practitioners lack sufficient knowledge about the diagnosis of the disease (36). Furthermore, the lab personals fail to follow SOPs regarding AFB smear microscopy, she argues that there is a crucial need to edify the health worker's circle (37). The temperature has a relation to TB, as the number of cases diagnosed peaks in summer. A study conducted in Lahore, Pakistan reported that the infection transmission was higher in winter, which lead to a higher number of patients diagnosed in summer (38). Two identified factors escalate the infection transmission in winter these are Vitamin D deficiency and overcrowded rooms (39). These factors are relatable to District Chiniot's environment as some areas are overcrowded.

The frequency of MDR-TB is increasing, according to WHO only half the patients diagnosed with MDR-TB are successfully treated (40). A study carried out in Congo from 2012-2017 has reported 11% RR-TB patients out of 1535 TB patients, in comparison to the current study in which the incident rate was 2% (41). A study reported in the light of their national survey 2012-2014, the Frequency of MDR-TB was 2.1% and the Frequency of RR-TB was 4.6% (42). The results of their study were higher than previously recorded data regarding RR-TB, which was 2.8% in 2001-2002, they extrapolated that the Frequency of RR-TB has doubled among new cases of TB (43). In India, the highest incident rate of MDR-TB is reported in the western states, which is 39.9% (44). Drug resistance in Pakistan is an emerging problem, A study conducted in Punjab, enlighten that 11.5% of isolates from TB patients were resistant to at least one drug, used to treat TB and 9.3% of isolates were multidrug-resistant (45). The present study provides evidence for the high incidence rate of TB and a substantial number of RR-TB in District Chiniot, Pakistan. While developed countries are on the verge to eradicate TB from their states, Pakistan is still overwhelmed by TB even though a lot of work is being done with the assistance of international funding bodies to eradicate TB in South Asia. However, a poorly executed program can lead to development of drug resistant strains. There is intense need to improve the quality of health by health departments in order to eradicate the TB and to get rid of resistance that is emerging in TB strains.

## CONCLUSION

The present study provides evidence for the high incidence rate of TB and a substantial number of RR-TB in males to females from district Chiniot Pakistan. While developed countries are on the verge to eradicate TB from their states, Pakistan is still overwhelmed by TB even though a lot of work is being

done, with the assistance of international funding. However, a poorly executed program can lead to the development of drug-resistant strains. We need to improve the quality of the health care system if we are to win this war against TB.

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