

Burden of Drug resistant Tuberculosis in newly diagnosed Tuberculosis patients of Khyber Pakhtunkhwa, Pakistan

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Abstract

Objective: To explore the burden of drug-resistant tuberculosis in Khyber Pakhtunkhwa, Pakistan.

Method: The cross-sectional study was conducted from March 1, 2016, to February 28, 2017, at the Khyber Pakhtunkhwa Tuberculosis Reference Laboratory, Hayatabad Medical Complex, Peshawar, Pakistan, and comprised referred suspected tuberculosis patient samples. Drug Susceptibility testing on all Mycobacterium tuberculosis complex strains was performed and data was subjected to statistical analysis.

Results: Of the 8220 samples, 4230 (51.5%) were related to females and 3990 (48.5%) to males. Also, 1978 (24%) were related to patients aged 15-24 years. Of the total, 1351 (16.5%) samples were positive on culture. Drug susceptibility testing showed 525 (39%) samples to be resistant to at least one of the first- and second-line drugs. Among the culture-positive cases, 5 (0.4%) were extensively drug-resistant, 62 (4.6%) multi-drug resistant, 243 (18%) polyresistant, 215 (16%) monoresistant and 826 (61%) were pan-sensitive.

Conclusion: Drug-resistant tuberculosis in newly-diagnosed tuberculosis patients was alarmingly high in Khyber Pakhtunkhwa region.

Keywords: Tuberculosis, MDR-TB, XDR-TB, Drug susceptibility test. (JPMA 71: 912; 2021)

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Introduction

Tuberculosis (TB) is caused by Mycobacterium tuberculosis complex (MTC).¹ Although there are more than 150 species of Mycobacterium, MTC is the most prevailing member responsible for 1.7 million deaths worldwide.² The World Health Organisation (WHO) declared TB as a global emergency in 1993.³ The WHO estimated 10.4 million new TB cases, including 490,000 multi-drug resistant-TB (MDR-TB) cases with five countries, India, China, Indonesia, Philippines and Pakistan, contributing 56% of the total TB burden. Pakistan is a high TB-endemic country with an estimated 518,000 cases, including 15,000 MDR-TB patients and stands 4th on the list of high MDR-TB burden countries. The estimated proportion of MDR-TB is 4% in new cases and 17.4% in patients who have previously been treated.² MDR-TB is still a global issue, including Pakistan, as only 47% cases are diagnosed due to constraints of limited resources of TB control Programmes and unavailability of drug susceptibility testing (DST).³ According to the drug resistance survey conducted in 2012 in Pakistan and some recent studies in the country, the prevalence of MDR-TB is in the range of 3.7% to 4.8% in newly-diagnosed TB cases, and 17.5% to 18.1% in previously treated TB cases.^{4,5}

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TB is transmitted as an infectious disease, and the predominant group for TB infections is 15-49 years.⁶ The use of anti-TB therapy in different combinations depends upon the situations, but in a standard regimen, isoniazid (INH), rifampicin (RIF), ethambutol (EBM) and pyrazinamide (PZA) are used daily for two months, followed by four months of INH and RIF given three times a week.² The therapeutic approach for drug-resistant tuberculosis (DR-TB) is complicated and requires a complex therapy for up to two years. The rapid emergence of drug resistance is a threat to global anti-TB efforts. MDR-TB is resistant to INH and RIF, while extensively drug-resistant TB (XDR-TB) is a type of MDR-TB which is additionally resistant to fluoroquinolone (FQ) and at least one of the second-line injectable drugs, like kanamycin (KAN), amikacin (AK) and capreomycin (CAP).⁷

Khyber Pakhtunkhwa (KP) is one of the provinces of Pakistan with 11.9% of the national population with an estimated 270 cases per 100000 population. Sputum-smear microscopy is used as an initial screening test for TB diagnosis, while Gen-eXpert assays are employed for the rapid detection of RIF-resistant TB (RR-TB) at the district level.⁸ MDR-TB is notified after a confirmatory drug susceptibility test (DST) at the central laboratory. Generally speaking, there is mostly incomplete reporting of DR-TB cases in KP and treatment continues without DST confirmation.

The current study was planned to establish the prevalence

of drug resistance in newly-diagnosed TB patients, especially those of MDR-TB and XDR-TB in KP.

Materials and Methods

The cross-sectional study was conducted from March 1, 2016 to February 28, 2017, at the KP Tuberculosis Reference Laboratory, Hayatabad Medical Complex, Peshawar, Pakistan, after approval from the ethics review committee of Kohat University of Science and Technology, Kohat, KP.

Pulmonary samples included sputum, gastric aspirate, bronchoalveolar lavage and gastric lavage/washing, while extra-pulmonary samples included pleural fluid, ascitic fluid, pus, cerebrospinal fluid (CSF), tissue biopsy, urine, pericardial fluid, lymph node, synovial fluid, lung biopsy, bone marrow and bone.

The samples was processed for fluorescent microscopy, culture and DST against first-line and second-line drugs, like RIF, INH, EBM, PZA, streptomycin (STR), moxifloxacin (MOX), AK, KAN, CAP and ofloxacin (OFX)⁹ (Sigma-Aldrich, St. Louis, MO, USA).

The samples were processed using N-acetyl-L-cysteine-Sodium hydroxide (NALC-NaOH) method for microscopy and culture in line with literature.¹⁰

Each sample was digested and decontaminated with NALC-NaOH in a biosafety level-III (BSL-III) laboratory. A drop of the processed sample was placed on the slide and fluorescent microscopy was done. Positive samples were confirmed using the Kinyoun modification of Ziehl Neelson (ZN) staining.

A total of 0.5ml decontaminated NALC-NaOH-processed specimens were used for culture on modified 7H9 middle brook media and incubated in Mycobacterium growth indicator tube (MGIT960) instrument (Becton Dickinson Diagnostic Instruments Systems, Sparks, MD, USA) with combination which is a combination of Poly-myxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocillin (PANTA). The positive culture from MGIT was then confirmed by TBc identification test (TBc ID) device through a chromatographic test detecting MPT64 protein.¹¹ The MPT64 is a mycobacterial protein secreted only by MTBC and has been shown to differentiate MTC from non-tuberculous mycobacteria (NTM).

Confirmed MTC isolates were processed for DST on MGIT960 against first-line drugs, like INH, with final critical concentration of 0.1 µl/ml, RIF with final critical concentration of 1.0 µl/ml, EBM with final critical concentration of 5.0 µl/ml, and STR with final critical concentration of 1.0 µl/ml, and against second-line drugs, like AK having critical concentration of 1.0 µg/ml, KAN

having critical concentration of 2.5 µg/ml, CAP having critical concentration of 2.5 µg/ml and OFX having critical concentration of 2.0 µg/ml. Data was subjected to statistical analysis.

Results

Of the 8220 samples, 4230 (51.5%) were related to females and 3990 (48.5%) to males. Also, 1978 (24%) were related

Table: Pulmonary and extra-pulmonary samples.

S. No.	Samples	Pulmonary	Extra pulmonary	Total n (%)
1	Sputum	7116	-	7116 (86.5)
2	Plueral Fluid	-	418	418 (5.1)
3	CSF	-	156	156 (1.8)
4	Ascitic fluid	-	188	188 (2.3)
5	Pus	-	81	81 (0.9)
6	Urine	-	44	44 (0.5)
7	Pericardial fluid	-	26	26 (0.03)
8	Gastric Aspirate	50	-	50 (0.6)
9	Lymph node	-	11	11 (0.1)
10	Synovial Fluid	-	8	8 (0.09)
11	Tissue Biopsy	-	75	75 (0.9)
12	Bronchoalveolar Lavage	28	-	28 (0.2)
13	Gastric Lavage/Washing	13	-	13 (0.3)
14	Lung Biopsy	-	3	3 (0.03)
15	Bone Marrow	-	2	2 (0.02)
16	Bone	-	1	1 (0.01)
	Grand Total	7207 (88%)	1013 (12%)	8220 (100)

CSF: Cerebrospinal fluid

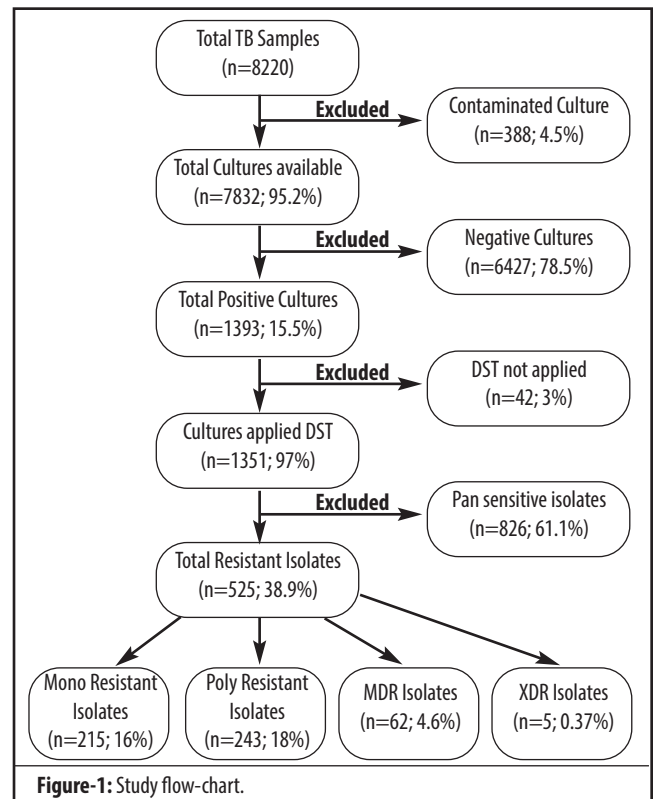
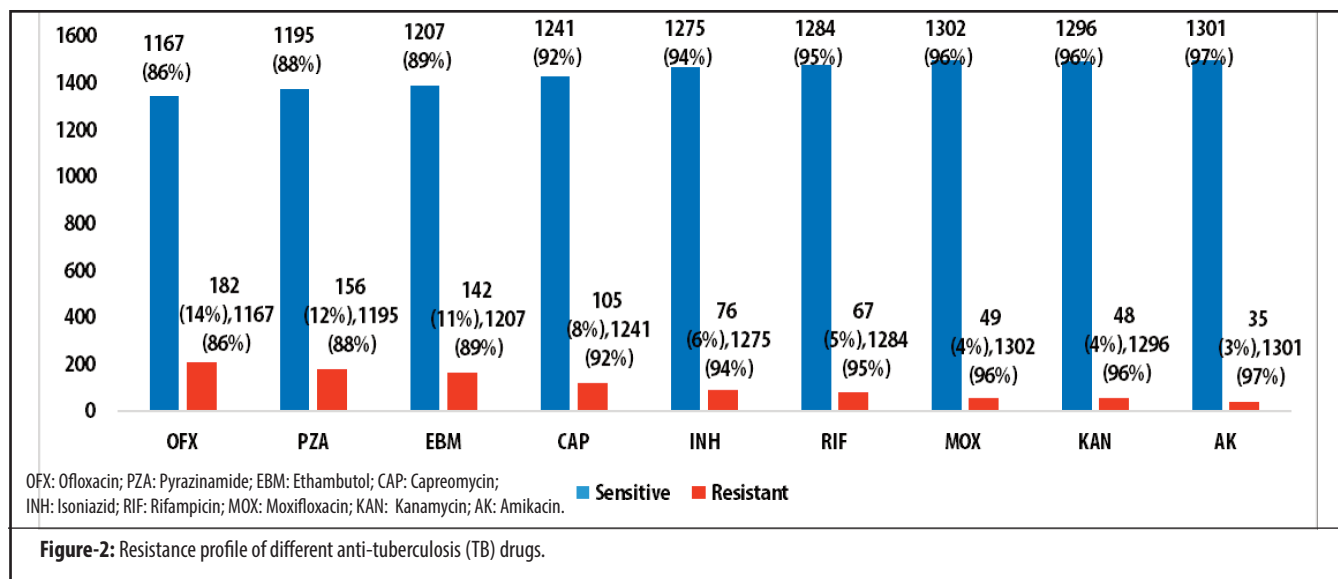


Figure-1: Study flow-chart.



to patients aged 15-24 years. There were 7207 (88%) pulmonary samples and 1013 (12%) were extra-pulmonary. The highest number of samples received were sputum 7116 (86.5%), while the lowest sample received was bone 1 (0.01%) (Table).

Of the total, 1351 (16.5%) samples were positive on culture. DST showed 525 (39%) samples resistant to at least one of the first- and second-line drugs. Among the culture-positive cases, 5 (0.4%) were XDR-TB, 62 (4.6%) were MDR-TB, 243 (18%) were polyresistant, 215 (16%) were mono-resistant and 826 (61%) were pan-sensitive (Figure 1). Of the 1351 culture-positive patients, 845 (61%) belonged to females and 548 (39%) to males. The age group most affected was 15-24 years with 472 (34.9%) cases. The least affected age group was >75 years 56 (4%). Among the first-line drugs, the most resistant was PZA 156 (12%), while the least resistant was RIF 67 (5%). Among the second-line drugs, OFX was the most resistant 182 (14%), while AK was the least resistant 35 (3%) (Figure 2).

Discussion

MDR-TB is an emerging global issue and, therefore, monitoring and surveillance are important factors in controlling TB.¹² About 18,422 cases of MDR-TB were reported in 2012 from 104 countries, and the toll increased to 490,000 cases in 2018.¹³ MDR-TB is a global issue as only 47% cases are diagnosed, and among all the registered MDR-TB cases, only 54% are treated successfully.⁸

The current study is the first to report preliminary data targeting new patients or those who did not receive any anti-TB drugs in the past. The new patients were checked for DR-TB, especially MDR-TB and XDR-TB, in the most war-affected province of Pakistan where a lot of internally

displaced persons (IDPs) and Afghan refugees are living and contributing almost 13% of the total TB burden in the country.⁴

The current study found about 4.5% MDR-TB prevalence, while earlier studies from Pakistan and KP have reported it to be 2-5%.^{14,15} The WHO global report 2018 stated that MDR-TB cases in new patients in Pakistan was 4.3%.² The current study also displayed higher rate from the first drug resistance survey (DRS) in Pakistan reporting it to be 3.7%.⁴ The incidence of MDR-TB in the current study is much less than 29% and 9% reported earlier from elsewhere in Pakistan.^{16,17} The reasons may be the inclusion-exclusion criteria and history of the patients.

The DR-TB develops due to inadequate treatment. This resistance can also develop due to poor treatment by medical practitioners. Resistance can be related to the linkage between human immunodeficiency virus (HIV) disease and drug resistant cases.¹⁸ In the current study, the 15-44 years age group was the most affected with TB, which is consistent with literature.^{3,6} It was also found that this age group was highly resistant to almost all antibiotics, especially OFX, PZA, EMB, CAP, INH and RIF. People developing MDR-TB in this age group has been reported by different national and international studies¹⁹⁻²¹ which may be explained in part by the fact that this age group is the most sexually active group. Though TB is not transmitted sexually, it can transfer with someone in close physical contact.

The current study found drug resistance was higher in females, which is in line with previous data from Pakistan²¹ and elsewhere.²² The current study, however, suggests that females were mostly infected while taking care of their

parents, husbands, children and even siblings. Besides, selection criteria may also have a role to play as more samples came from females.

In the current study, the prevalence of MDR-TB was 4.5%, but studies show different reports in different geographical areas, like 2.8% in China²³ and 4.7% in Brazil.²⁴ These differences may be due to study designs, living conditions, study inclusion-exclusion criteria and other socio-economic issues.

Against second-line drugs the highest resistance was seen in OFX 14%, while the least resistance was against AK 3%. An earlier study showed AK to be the least resistant 7%, while amikacin (AMK) was the most resistant 44%.²⁵

Future studies should also investigate the drug resistance mechanism of MTC at the molecular level.

Conclusion

There was a high prevalence rate of MDR-TB and XDR-TB in new patients, which is a matter of great concern. Timely diagnosis of such cases will be helpful in controlling TB through appropriate treatment.

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Conflict of interest: None.

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