

Prevalence of Multi-Drug Resistant *Mycobacterium Tuberculosis* in Khyber Pakhtunkhwa – A High Tuberculosis Endemic Area of Pakistan

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Abstract

Anti-tuberculosis therapy involves the combination of drugs to hamper the growth of *Mycobacterium tuberculosis* (MTB). The emergence of multidrug-resistant tuberculosis (MDR-TB) is a global concern. Pakistan has been ranked 5th position in terms of a high burden of MDR-TB in the world. The aim of the current study was to investigate the prevalence of drug resistance in MTB in Khyber Pakhtunkhwa. Random samples were collected from 25 districts using the simple random sampling formula. All samples were processed in a biosafety level 3 laboratory for culture and drug susceptibility testing. Among 5759 presumptive tuberculosis (TB) cases, 1969 (34%) were positive. The proportion of TB was higher in females (39%) than males (29%), thus it represents a significant association between gender and tuberculosis ($p < 0.05$). People ages between 25 to 34 years were more likely to be infected with MTB (40%). Drug-resistant profile showed 97 (4.9%) patients were infected with MDR-TB. Streptomycin resistance was the highest and was observed in 173 (9%) isolates followed by isoniazid in 119 (6%) isolates. The lowest resistance was observed to pyrazinamide (3%). The prevalence of MDR-TB (10.4%) among patients that previously received anti-tuberculosis treatment is seemingly high. A large-scale drug resistance survey is required to evaluate the drug resistance for better management of tuberculosis.

Key words: tuberculosis, MDR MDR-TB, multi drug-resistant TB

Introduction

Tuberculosis (TB) is a pre-historic disease caused by *Mycobacterium tuberculosis* (MTB) (Daniel 2006). Although there are more than 150 species of *Mycobacterium* sp., MTB is still the most dominant and prevailing member of this genus all over the world, accounting for 10 million deaths in 2019 (WHO 2019).

The World Health Organization (WHO) declared TB as a global emergency in 1993 (Grange and Zumla 2002). Despite significant medical and social interventions, TB consistently affects vulnerable populations across the world and remains a leading global public health problem. Treatment of drug-susceptible TB takes six months while treatment of rifampicin-resistant

TB (RR-TB) and multidrug-resistant TB (MDR-TB) requires a long therapy for up to two years (WHO 2019).

Globally, an 85% successful treatment rate has been reported for drug-susceptible MTB. The emergence of drug resistance, however, still poses a threat to global efforts. The WHO estimated 10.4 million new TB cases consist of 490 000 multidrug-resistant TB and 110 000 rifampicin-resistant TB. Five countries such as India, China, Indonesia, Philippines, and Pakistan are accounting for 56% of TB burden. Despite the development of rapid molecular tools, only 37% of MDR-TB were reported globally which shows laboratory gaps. China, India, and Russia reported 47% of the total global MDR/RR-TB cases. Pakistan is a high TB endemic country, standing at 5th position in the list

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of 30 high burden countries (HBC) with an estimated 518 000 TB cases including 15 000 MDR-TB. The estimated proportion of MDR-TB is 4.2% in new patients and 16% in the previously treated patients (WHO 2019). According to the drug resistance survey conducted in 2012, the prevalence of MDR-TB was 3.7% in newly diagnosed TB cases and 18.1% among previously treated TB cases (Tahseen et al. 2016). KPK is one of the four provinces of Pakistan that contributes a proportion of 11.9% in the total national population with an estimated 270 TB cases per 100 000 population (NTP 2014). Patients with drug-susceptible TB receive anti-TB treatment for at least six months while patients with MDR-TB and RR-TB receive longer treatments comprising of second-line drug regimens (NTP 2015). The sputum smear microscopy is used as an initial screening test for TB diagnosis, while GeneXpert assays are employed for the rapid detection of RR-TB at the district level (NTP 2015). In the current study, we analyzed the prevalence of MDR-TB among different lineages prevalent in the Khyber Pakhtunkhwa (KPK) province of Pakistan. MDR-TB is notified after a confirmatory DST test performed at the central BSL-III laboratory.

Experimental

Material and Methods

Study site. Random samples were collected from 25 districts of KPK using a simple random sampling (SRS) formula, which was previously used in a national TB survey of Pakistan:

$$n(SRS) = \frac{N \times z^2 \times p \times (1-p)}{d^2 \times (N-1) + z^2 \times p \times (1-p)}$$

where

SRS – Sample Random Sampling

N – total number of new smear positive cases registered in the lab

z – z-value (from the standard normal distribution) that corresponds to the desired confidence level

d – absolute precision

p – expected proportion of MDR patient in the target population

Study participants and sample collection. A total of 1969 positive *M. tuberculosis* cases were calculated using this SRS formula. To achieve the target samples a total of 5759 clinical samples were collected from 25 districts and were diagnosed for TB. The patients' data were collected from their parents or the next caretakers.

Sputum processing. All received samples were digested and decontaminated using standard N-acetyl-L-cysteine sodium hydroxide (NALC-NaOH) method (GLI 2014) in a biosafety level 3 laboratory (BSLIII)

at the Provincial TB Reference Laboratory, Peshawar. Briefly, one aliquot was inoculated on the Lowenstein Jensen medium (LJ) and in a Mycobacterium growth indicator tube (MGIT). Positive growth in the tubes was confirmed by Tbc ID device (Ref: 245159, Becton, Dickinson).

Drug susceptibility testing (DST). All confirmed mycobacterial isolates were processed for both phenotypic DST and molecular resistance assay. DST was performed using a BD BACTEC MGIT 960 SIRE kit (Ref: 245123, Becton, Dickinson), in which the final drug concentration was 1 µg/ml for RIF, and 0.1 µg/ml for INH. One sample aliquot was processed for acid-fast bacilli (AFB) microscopy using Primostar-LED fluorescent microscopy.

Data analysis. Results were recorded in the local laboratory management information software and analyzed using SPSS V.15 (IBM, USA). Sensitivity and specificity were calculated using Medcalc software (<https://www.medcalc.org>).

Results

Among 5759 TB suspects, 1969 (34%) were culture-positive, 3121 (54%) were culture-negative, and 344 (6%) were contaminated. The proportion of TB was higher in females (39%) than males (29%), thus, a strong association was observed between the gender and tuberculosis disease ($\chi(3) = 68.2, p = 0.001$). It was observed that the age group of 25–34 years was more likely infected with TB (40%) when compared to other groups (Table I). The susceptibility testing towards the first-line drugs as rifampicin, isoniazid, ethambutol, streptomycin, and pyrazinamide was performed on 1969 culture-positive isolates.

DST results of 1969 isolates showed that 238 (12%) isolates were resistant to at least one drug, while 97 (4.9%) were confirmed to be MDR-TB. The remaining 1731 (88%) isolates were sensitive to all drugs. The drug resistance was the highest to streptomycin in 173 (9%) isolates, followed by isoniazid in 119 (6%), ethambutol in 101 (5%), rifampicin in 99 (5%), and pyrazinamide in 65 (3%) isolates.

The drug resistance found was correlated with different factors from the patient history including age, gender, and treatment history. MDR was observed in 61 (5.2%) males and 36 (4.5%) in female patients. No significant association of MDR with gender ($\chi(1) = 1, p\text{-value} = 0.26$) or age group ($\chi(5.8) = 6, p\text{-value} = 0.44$) was observed. The prevalence of MDR was higher in the age group of 55–64 years (6.4%), followed by a group of 15–24 years (6%) (Table II). MDR correlation with pulmonary and extra-pulmonary TB was also analyzed and it was found that the prevalence of MDR in pulmonary

Table I
Association of TB with gender, different age groups and treatment history, *p*-value of < 0.05 shows a statistical significance.

Character	TB Suspects	Positive cases	<i>p</i> -value
Gender			
Male	3189	947 (29%)	$\chi(3) = 68.2, p = 0.005$
Female	2570	1022 (39%)	
Age group			
01–14	437	96 (22%)	
15–24	1137	396 (34%)	
25–34	1180	473 (40%)	
35–44	827	298 (26%)	
45–54	732	227 (31%)	
55–64	819	282 (34.4%)	
65–100	627	197 (31.4%)	
Treatment history			
Previously Treated	1024	461 (45%)	$\chi(3) = 68, p\text{-value} = 0.05$
Never Treated	3922	1508 (38%)	
Disease type			
Pulmonary	5290	1864 (35%)	$\chi(3) = 68, p\text{-value} < 0.05$
Extra Pulmonary	469	105 (22%)	
Sample type			
Ascetic Fluid	91	10 (11%)	$\chi(36) = 259.6, p\text{-value} \leq 0.05$
BAL*	172	45 (26%)	
Bone	20	2 (10%)	
CSF**	44	2 (5%)	
Gastric Lavage	68	5 (7%)	
Lymph Node	10	3 (30%)	
Pericardial fluid	26	3 (12%)	
Pleural Fluid	172	26 (15%)	
Pus	54	9 (17%)	
Sputum	5033	1858 (37%)	
Synovial Fluid	3	0 (0%)	
Tissue	20	5 (25%)	
Urine	46	1 (2%)	

* Bronchoscopy alveolar lavages, ** Cerebrospinal fluid

TB was significantly higher 94 (5.3%) when compared to extra-pulmonary TB (1.5%), $\chi(5.3) = 1, p\text{-value} = 0.009$. The resistance of the MTB isolates from the previously treated patients was significantly higher in 48 (10.4%) cases when compared to 49 (3.2%) untreated patients. It can indicate an association of drug resistance with the patient treatment history ($\chi(16) = 2, p\text{-value} = 0.001$).

Discussion

MDR-TB is a major threat to public health. Monitoring its trends over time is crucial to prevent further emergence of drug resistance. Surveillance of drug

resistance is, therefore, a critical component of any TB control Programme (Zignol et al. 2016). A decade back, only 18 422 laboratory-confirmed MDR-TB cases were reported from 104 countries. It escalated to an estimated 490 000 cases in 2016 (WHO 2019). Even today MDR-TB is a persistent threat to the global community but unfortunately, only 47% of MDR cases could be diagnosed among the global estimates due to limited resources and laboratory gaps. Similarly, among all the registered MDR-TB cases, only 54% could be successfully treated. This study provides preliminary data of MDR-TB in KPK, which contributes to 13% of the national TB burden. In this first large-scale data, we found that MDR-TB was detected among 4.9% of

Table II
Correlation of the MDR-TB prevalence with patient's age, gender,
and previous treatment history.

Character	Total DST	Diagnosed with DR-TB	<i>p</i> -value
Gender			
Male	1167	61 (5.2%)	$\chi(1) = 1, p = 0.26$
Female	800	36 (4.5%)	
Age group			
01–14	186	5 (2.6%)	$\chi(5.8) = 6, p = 0.445$
15–24	333	20 (6%)	
25–34	372	18 (4.8%)	
35–44	279	11 (4%)	
45–54	258	10 (3.8%)	
55–64	294	19 (6.4%)	
65–100	245	14 (5.7%)	
Treatment history			
NT*	1508	49 (3.2%)	$\chi(16) = 2, p\text{-value} = 0.001$
PT**	461	48 (10.4%)	
Disease type			
Pulmonary	1771	94 (5.3%)	$\chi(5.3) = 1, p\text{-value} = 0.009$
Extra Pulmonary	196	3 (1.5%)	

* NT = Never treated; ** PT = previously treated; DST = drug susceptibility testing;
DR-TB = drug resistant tuberculosis

newly diagnosed patients. This figure is consistent with the first national DRS of Pakistan where it has been reported 3.7% MDR-TB cases (Tahseen et al. 2016) and 3.6% the global estimates (WHO 2019). Similar findings from Pakistan reported a 2–5% MDR-TB ratio (Javaid et al. 2008; Ejaz et al. 2010). A comparative high ratio of 29% and 9% MDR-TB was reported in early literature from other areas of Pakistan (Javaid et al. 2016; Shah et al. 2016). However, Akhtar et al. demonstrated a much higher MDR ratio of 69% in a study performed in Punjab (Akhtar et al. 2016). Possible differences in these reports might be due to the variance in study design and sample inclusion criteria. People ages 15 to 34 years old were at high risk to develop MDR-TB (Hoa et al. 2015; Akhtar et al. 2016; Khan et al. 2018). The increased drug resistance in previously treated cases (10.4%) was high as compared to newly diagnosed patients. These findings are consistent with previously published data (Tahseen et al. 2016). TB has been found to be more prevalent in males (Neyrolles and Quintana-Murci 2009); however, we did not detect a significant correlation of MDR-TB with gender, extra-pulmonary TB or a sample type (Bhattacharjee and Datta 2014; Wattal et al. 2015). In contrast to this, data from Africa shows a relatively high prevalence of drug-resistant TB in women (O'Donnell et al. 2011).

In conclusion, MDR-TB is an emerging problem in Khyber Pakhtunkhwa, Pakistan. This study has highlighted the MDR surveillance among the population

of a geographically distinct area of Pakistan. Knowing the approximate magnitude of MDR-TB, this study will help for better management of drug resistance towards global TB control 2030.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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