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Drug resistance profile of *Mycobacterium tuberculosis* and predictors associated with the development of drug resistance

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ABSTRACT

Objectives: Drug-resistant tuberculosis (DR-TB) is a major challenge to national TB control programmes in developing countries. In the Pakistan province of Punjab, the extent and development of DR-TB is not well known. The current study was therefore conducted to assess the incidence and predictors of DR-TB in Punjab Province.
Methods: Drug susceptibility testing was performed for 863 confirmed culture-positive *Mycobacterium tuberculosis* isolates using the proportion method. Patients were enrolled in the Programmatic Management of Drug-Resistant TB Unit of Gulab Devi Chest Hospital (Lahore, Pakistan) from August 2011 to September 2013. Data analysis was performed using IBS SPSS Statistics v.20. Multivariate logistic regression analysis was performed to assess risk factors for DR-TB.
Results: The rate of resistance to at least one drug (i.e. DR-TB) was 35.0% (302/863) and the rate of multidrug-resistant TB (MDR-TB) was 30.0% (259/863). Among DR-TB cases, the number of females was relatively higher (167/302; 55.3%) compared with males. The majority of DR-TB patients resided in a rural area (229/302; 75.8%). Significant predictors of DR-TB were age 18–45 years, previous TB treatment, rural residence, being a housewife, being married, duration of sickness >1 year and unemployment.
Conclusion: The problem of DR-TB in Pakistan is significant. The strongest risk factors were young age and previous anti-TB treatment. Being married, being a housewife, rural residence and unemployment were also risk factors, culminating in an urgent need for effective control, early diagnosis and treatment policies for DR-TB.

1. Introduction

The aim of slowing down the spread tuberculosis (TB) has achieved limited success despite the application of TB control strategies such as timely detection of TB and the directly observed treatment, short-course (DOTS) strategy [1]. Globally, the control of TB and human immunodeficiency virus (HIV) co-infection has become problematic, causing the rise of more multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB cases [2].

For TB control, it is crucial to consider the composite risk factors and socioeconomic factors associated with the extent of TB infection at the population level [3]. Better commitment of TB control programmes has encouraged discussion about the social factors associated with TB, inspired by the constantly growing number of TB cases and the unequal spreading all over the world [4].

The highest number of TB cases were detected in 2010 than ever before in human history and these were in disadvantaged groups of poverty-stricken, deprived traditional minorities [4]. Many studies have concentrated on the outcome of HIV/TB co-infection, but limited data are available on the impact of socioeconomic status as

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well as behavioural aspects in the occurrence of TB and the emergence of drug-resistant TB (DR—TB) in an endemic area such as Pakistan.

In 2104, there were approximately 9.4 million new cases of TB along with 1.5 million deaths due to the disease, demonstrating that globally TB is a major social health problem [5]. Pakistan is ranked fifth in the list of high-burden TB countries, with prevalences of 270 smear-positive TB cases per 100 000 population and 398/100 000 bacteriologically positive TB cases [5]. With approximately 4.2% resistance in untreated TB cases and 19% in previously treated patients, Pakistan ranks fourth among 22 countries with alarmingly increasing MDR-TB [6].

The increase of MDR-TB (resistant to at least rifampicin and isoniazid) along with relapse, failure of TB treatment, complications and deaths are creating a hurdle in controlling the spread of TB [7,8]. The response of MDR-TB patients to first-line anti-TB regimens such as pyrazinamide and ethambutol is not appreciable and ultimately results in a high mortality rate. Treatment of such patients using second-line regimens is not only costly and lethal but also requires hospitalisation of the patient to deal with complications that are caused as a side effect of treatment [7,9,10]. Therefore, in developing countries during the management of MDR-TB cases, some additional restrictions are likely on healthcare resources [11,12]. The reduced efficacy of accessible anti-TB regimens against XDR-TB further makes it problematic to effectively treat TB [13]. One of the main reasons for drug resistance in TB is inadequate compliance with the DOTS guidelines and insufficient attention to the TB patient [7,8]. Limited research has been performed in laboratory settings on DR-TB and, moreover, there is limited information regarding the prevalence of and risk factors for MDR-TB in Pakistan. Thus, information from the present study will not only be valuable to determine the extent of the problem in a high-risk population but may also help the TB control programme to establish essential steps that can halt the spread of MDR-TB in the country. The purpose of this study was to report the incidence of DR-TB from Punjab Province, Pakistan, a highly TB-endemic area, and to evaluate the impact of different risk factors for DR-TB.

2. Materials and methods

2.1. Study setting

The current research work was carried out at the Programmatic Management of Drug-Resistant TB (PMDT) Unit of Gulab Devi Chest Hospital in Lahore, Pakistan. It is the largest PMDT Unit in Punjab Province, covering more than 32 TB-endemic districts in the province. Punjab is the largest province of Pakistan, with an estimated population of 101 million and an area of 205 344 km^2.

2.2. Study population

A total of 901 culture-positive suspected DR-TB patients were enrolled in this study from August 2011 to September 2013. All pulmonary DR-TB patients, irrespective of age and sex, were analysed. Cultures on which drug susceptibility testing (DST) could not be applied as well as nontuberculous mycobacteria were excluded from the final analysis.

2.3. Data collection method

A self-designed proforma was used to collect the history of the participants and also by interview and looking at the medical profile of the patients. The feedback form comprised information related to clinical, sociodemographic, socioeconomic status and microbiological data. During interpretation of the results, smoking was taken as a single variable whether it was in active form or was done in the past by the patient. The collected history of the patients comprised the following clinical and environmental parameters: sex; age; area of residence (rural/urban); economic status/occupation; when TB was first diagnosed; information of previous anti-TB treatment; family history of TB; and smoking.

2.4. Laboratory methods

2.4.1. Clinical samples

Clinical samples consisted of 863 positive cultures from suspected DR-TB patients. One specimen was collected from each enrolled case. The specimen was used for smear microscopy, culture and DST. Newly diagnosed cases were those with no history of anti-TB treatment, whereas defaulters or those having previously received anti-TB treatment were labelled as previously treated cases).

2.4.2. Decontamination and processing for culture

The *N*-acetyl-L-cysteine sodium hydroxide (NALC-NaOH) method was used for decontamination of the specimens followed by 15 min of centrifugation (3000 × *g*), and the sediment was used for smear and culture [14–16]. Culture was performed on Löwenstein–Jensen (LJ) medium (Oxoid) and also using a BD BACTEC™ MGIT™ 960 system (Becton Dickinson Diagnostic Systems, Sparks, MD). Briefly, 0.2 mL of the concentrated specimen was inoculated on an LJ slant and was incubated until positive growth or for up to 8 weeks. Also, 0.5 mL of the specimen was inoculated in MGIT vials and was incubated at 37 °C following supplementation of the medium with PANTA antibiotic supplement comprising amphotericin B, polymyxin B, trimethoprim, nalidixic acid and azlocillin. Inoculated vials were examined to determine the growth index after 8 weeks or when there was positive growth.

2.4.3. Identification of Mycobacterium tuberculosis

Positive growth obtained from the LJ slant and MGIT vials was stained using Kinyoun stain [6] and *M. tuberculosis* isolates were identified through the differentiation test of BACTEC NAP TB (Becton Dickinson). Niacin positivity and nitrate reduction were also used for differentiation [7].

2.4.4. Drug susceptibility testing of Mycobacterium tuberculosis *isolates*

The agar proportion method was used for DST, which was based on enriched Middlebrook 7H10 medium (BBL) comprising the following concentrations: 1 μg/mL and 5 μg/mL rifampicin; 0.2 μg/mL and 1 μg/mL isoniazid; 2 μg/mL and 10 μg/mL streptomycin; and 5 μg/mL and 10 μg/mL ethambutol [6,17]. BACTEC 7H12 medium (pH 6.0) (Becton Dickinson) was used to determine susceptibility to pyrazinamide [6,17]. In each batch of DST, *M. tuberculosis* H37Rv was used as a control strain. Resistance of *M. tuberculosis* strains to at least the two most important first-line regimens (isoniazid and rifampicin) is known as MDR-TB, and XDR-TB strains are MDR-TB strains also resistant to any fluoroquinolone and at least one second-line injectable drug among kanamycin, capreomycin or amikacin. DR-TB was considered as resistance to one or more first-line anti-TB drugs. A patient failure case was defined as re-initiation of an anti-TB treatment regimen following the non-success of previous anti-TB therapy. A relapse case was defined as a previously treated patient who was stated as cured after completion of treatment but who was bacteriologically positive after culturing or smear microscopy.

2.5. Analysis and data management

Data were entered into Microsoft Excel (Microsoft Corp., Redmond, WA) and were interpreted using IBM SPSS Statistics v.20 (IBM Corp., Armonk, NY). The mean and standard deviation were calculated for normally distributed quantitative variables, whereas the median and interquartile range were calculated for quantitative variables with unidentified distribution. Frequency and percentage (%) were calculated for categorical variables. The prevalence was independently calculated for all of the DR-TB isolates. The χ^2 test of independence was used to estimate the link among the status of DR-TB and entire categorical patient and factors. When there was a significant link ($P < 0.05$) between χ^2 test and *t*-test, then the direction of the association was calculated by logistic regression. Univariate analysis was performed using the χ^2 test. Logistic regression analysis was used for the significantly associated risk factors in the univariate analysis ($P < 0.05$).

2.6. Ethical approval and consent to participate

This research work was approved by the Ethical Committee of the University of the Punjab in accordance with the ethical standards of the responsible committee on human experimentation and with the latest (2008) version of the Declaration of Helsinki [18]. The purpose of the study was explained, and written consent was obtained from all patients or their guardians or from the next of their kin, caretakers or guardians/parents of all child participants.

3. Results

Of 901 positive cultures, 25 were found to be nontuberculous mycobacteria and DST could not be performed on 13 isolates, which were therefore excluded; thus, a total of 863 culture-positive patients were included in the final analysis. The prevalence of resistance to at least one drug (i.e. DR-TB) was 35.0% (302/863) and that of MDR-TB was 30.0% (259/863). Among DR-TB patients, the number of females (167/302; 55.3%) was higher than that of males. The mean age of DR-TB patients was 31.03 ± 14.78 years (range 10–90 years). The majority of patients resided in a rural area (229/302; 75.8%). Other sociodemographic characteristics are detailed in Table 1.

3.1. History of patients with drug-resistant tuberculosis

Information about the medical history of patients with DR-TB is presented in Table 1. Approximately one-third of the patients (96/302; 31.8%) had a duration of sickness of >1 year before receiving a confirmed diagnosis of DR-TB. The duration varied between 3 weeks and >1 year. A small proportion of patients (15/302; 5.0%) also reported a history of co-morbidities. The most common co-morbidity was diabetes (10 patients), followed by hypertension and renal disease in 1 patient each. A total of 269 patients (89.1%) also reported a previous history of TB that was treated with anti-TB drugs.

3.2. Drug resistance

Of the 302 DR-TB cases, most of the patients were suffering from MDR-TB (259/302; 85.8%). XDR-TB was present in 14 cases (4.6%), whilst polydrug-resistant and monoresistant TB were present in 10 (3.3%) and 19 (6.3%) cases, respectively. Patients were found to be resistant to a mean of 4.30 ± 0.93 first-line drugs (range 1–5). The most common resistance was found to rifampicin (100%) and isoniazid (92.9%), followed by pyrazinamide (82.5%), ethambutol (74.2%) and streptomycin (65.6%).

3.3. Factors associated with drug-resistant tuberculosis

Table 2 shows the results of the logistic regression to evaluate significant predictors of DR-TB. Univariate analysis revealed that

Table 1
Sociodemographic characteristics and medical and disease history of patients with drug-resistant tuberculosis (DR-TB) ($N = 302$).

Variable	*n* (%)
Sex	
Male	135 (44.7)
Female	167 (55.3)
Marital status	
Unmarried	86 (28.5)
Married	216 (71.5)
Age	
<18 years	43 (14.2)
18–45 years	188 (62.3)
46–60 years	54 (17.9)
>60 years	17 (5.6)
Employment status[a]	
Employed	31/136 (22.8)
Unemployed	36/136 (26.5)
Housewife	52/136 (38.2)
Student	17/136 (12.5)
Residence	
Urban	73 (24.2)
Rural	229 (75.8)
Duration of sickness	
≤1 year	206 (68.2)
>1 year	96 (31.8)
Co-morbidities	
No	287 (95.0)
Yes	15 (5.0)
Previous history of TB	
Positive	269 (89.1)
Negative	33 (10.9)
Smoking history	
No	246 (81.5)
Yes	56 (18.5)

[a] Data were not available for 166 patients.

Table 2
Factors associated with drug-resistant tuberculosis (DR-TB) ($N = 302$).

Predictor	*n* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Sex			
Male	135 (44.7)	1	
Female	167 (55.3)	0.85 (0.58–1.23)	1.10 (0.66–1.85)
Residence			
Urban	73 (24.2)	1	
Rural	229 (75.8)	1.97* (1.20–3.25)	1.69 (0.98–2.92)
Age			
<18 years	43 (14.2)	1	
18–45 years	188 (62.3)	6.53* (2.64–16.15)	3.91* (1.17–13.02)
46–60 years	54 (17.9)	1.83 (0.95–3.56)	0.96 (0.34–2.67)
>60 years	17 (5.6)	0.91 (0.51–1.63)	0.44 (0.19–1.04)
Marital status			
Unmarried	86 (28.5)	1	
Ever married	216 (71.5)	2.06* (1.29–3.27)	1.86 (0.89–3.91)
Employment status[a]			
Employed	31/136 (22.8)	1	
Unemployed	36/136 (26.5)	2.95* (1.09–7.97)	
Housewife	52/136 (38.2)	2.87* (1.10–7.49)	
Student	17/136 (12.1)	1.67 (0.47–5.94)	
Smoking history			
No	246 (81.5)	1	
Yes	56 (18.5)	0.97 (0.59–1.59)	0.98 (0.51–1.89)
Duration of sickness			
≤1 year	206 (68.2)	1	
>1 year	96 (31.8)	2.03* (1.37–2.99)	1.30 (0.81–2.09)
Co-morbidities			
No	287 (95.0)	1	
Yes	15 (5.0)	0.85 (0.34–2.13)	0.69 (0.25–1.91)
Previous history of TB			
Positive	269 (89.1)	8.875 (5.88–14.62)	8.90* (5.57–15.59)
Negative	33 (10.9)	1	

OR, odds ratio; CI, confidence interval.
[a] Data were missing for 166 patients, thus this variable was not included in the final model.
* Statistically significant association ($P \leq 0.05$).

females (55.3%) were relatively more likely to experience DR-TB compared with males (44.7%); however, the association was not significant ($P > 0.05$). The presence of co-morbidities and smoking history were also not significantly related to the presence of DR-TB. Compared with employed patients, being unemployed and being a housewife was found to be significant risk factors for DR-TB [crude odd ratio (OR) = 2.95 and 2.87, respectively]. Rural residents, married residents and those with >1 year of sickness were independently related to DR-TB (Table 2) during the univariate analysis; however, the association disappeared in the multivariate analysis.

Two significant predictors of DR-TB were found during multivariate logistic regression, namely age 18–45 years and previous TB treatment (Table 2). Patients aged 18–45 years were almost four times more at risk of DR-TB compared with patients aged >60 years [adjusted OR = 3.91, 95% confidence interval (CI) 1.17–13.02]. Patients with a history of previous treatment with anti-TB drugs were almost nine times more likely to experience DR-TB compared with patients having no history of anti-TB treatment (adjusted OR = 8.90, 95% CI 5.57–15.59) (Table 2).

4. Discussion

Here we present finding on the prevalence of and risk factors associated with DR-TB in the largest and highly TB-endemic area of Punjab, Pakistan. The results of this study showed that 89.1% of DR-TB patients had previously received treatment and the remaining 10.9% were newly diagnosed. Age 18–45 years, previous anti-TB treatment, rural residence, being married, being a housewife, duration of sickness >1 year and unemployed status were significant risk factors for DR-TB.

In this study, the prevalence of drug resistance in previously treated patients was greater than in newly diagnosed patients, as previously reported elsewhere in Pakistan [15,19,20]. The high rate of DR-TB in previously treated cases compared with newly diagnosed cases is reported by many other studies in other countries [21–23]. From the current study, we suggest that previous treatment is inadequate and poses a risk to continued TB transmission in the Pakistan community. The prevalence of resistance to at least one drug was 35.0% (302/863), whilst the prevalence of MDR-TB was 30.0% (259/863). In this study, the prevalence of MDR-TB was 27% in previously treated patients and 3% in newly diagnosed patients, which is line with previous studies in Pakistan [19,20,23] and worldwide [23,24]. The current study validates the results of other studies such as those by Daniel and Osman [25], Faustini et al. [26] and Lomtadze et al. [27], who reported that a risk factor for MDR-TB is previous TB treatment. The prevalence of MDR-TB from different areas of the world ranges from 12 to 37% in previously treated patients, whilst in newly diagnosed cases it ranges from 2.1 to 12% [28]. The MDR-TB prevalence in Japan has been reported as 0.7% in newly diagnosed patients and 9.8% in previously treated patients [29], and in China the rates of MDR-TB were 2.8% and 14.7% in new and previously treated patients, respectively [24]. Micheletti et al. reported 2.2% MDR-TB in newly diagnosed patients and 12% in previously treated patients from Brazil [30]. Differences in the prevalence of MDR-TB in different areas may be due to different living standards, TB control programmes, socioeconomic factors and healthcare provision. Most cases of MDR-TB in previously treated patients are indicators of the deficiency of treatment supervision, poor patient compliance and ineffective national TB control programmes (NTCPs) in a highly endemic country such as Pakistan, and new cases may be due to transmission of DR-TB in the population.

The association between MDR-TB and age is not well established because different researchers have used different age group cut-offs in their studies. However, it has been reported that MDR-TB patients are more likely to be younger [19,23]. Another important risk factor in the current study was age. Patients aged 18–45 years were almost four times more likely to experience DR—TB compared with patients aged >60 years, which is similar to other studies [19,23,26,31–33]. Possible reasons for the age-related differences may be that patients aged 18–45 years are often busy in routine activities such as study, work in an office or other undertakings such as earning for their family in comparison with older people whose lifestyle is inactive [19,23,31].

The association of sex with DR-TB differs in different areas. In the current study, females were relatively more likely to experience DR-TB compared with males; however, the association was not significant, which is line with other studies [19,25]. Lomtadze et al. [27] and Ejaz et al. [34] also reported that female sex was a risk factor for MDR-TB, however in other studies male sex was a greater risk factor [35,36].

In this study, unemployed status in comparison with employed status was also found to be a significant risk factor for the development of DR-TB (OR = 2.95), which is in line with previous studies elsewhere [37,38].

In this study, being a housewife was found to be a significant risk factor for DR-TB (OR = 2.87). We hypothesised that the role of housewives in Pakistan as caregivers may have made them prone to developing DR-TB as they have more contact with MDR-TB patients compared with males at home. Another possible reason may be that housewives in Pakistan are busy in their homes taking care of their families and could lack time, making it difficult to thoroughly manage their medication.

In this study, rural residence was independently related to DR-TB in the univariate analysis. It may be that rural area patients belonged to remote areas with a low level of education, poor socioeconomic conditions, inadequate health facilities, malnutrition and inadequate implementation of DOTS. Education and counselling may play an important role in decreasing and preventing MDR-TB in rural areas.

In the current study, the presence of co-morbidities and smoking history were not significantly related to DR-TB. These finding are in contrast to other studies in which smoking and co-morbidities (diabetes mellitus) were associated with MDR-TB [39,40].

In Pakistan, culture and DST laboratories are in limited number for the diagnosis of DR-TB cases. However, the laboratory challenge can be overcome quickly by the introduction of rapid diagnostic techniques such as Xpert MTB/RIF, which can detect *M. tuberculosis* and rifampicin resistance within 2 h. This rapid diagnostic technique can at least make a diagnosis of MDR-TB within 2 h and standardised treatment can be initiated for patients while awaiting DST results [19]. In the near future, all new TB suspected patients should be screened by this fast diagnostic test to decrease or halt the transmission of disease from infected person to others in the community. Although this fast diagnostic test cannot replace conventional methods (culture and DST), it can be significant in the rapid scale-up of laboratory facilities in developing countries such as Pakistan where essential biosafety standards and cost may be limiting factors.

Limitations of this study are the small sample size and the highly selected DR-TB suspected patients referred from hospitals or clinics to the PMDT Unit. Thus, the results cannot be applied to the general population. Despite these limitation, this study provides useful information in determining a resistance profile and risk factors of *M. tuberculosis*.

5. Conclusion

Previous TB treatment and age 18–45 years were the strongest risk factors associated with DR-TB. More and detailed studies of the reason for poor treatment efficacy could help progress in control policies. The rise of MDR-TB, especially in young and previously treated patients, highlights the urgent requirement to support NTCPs in highly TB-endemic countries such as Pakistan. Therefore, with knowledge of these risk factors, there is an urgent need for early detection of DR-TB, and care of TB patients with known risks needs to be the first priority to reduce MDR-TB in Pakistan.

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Competing interests

None declared.

Ethical approval

This study was approved by the Ethical Committee of the University of Punjab (Lahore, Pakistan).

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