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# Pattern of drug resistance in multi drug resistant tuberculosis patients in Punjab, Pakistan

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## Abstract

The prevalence rate of tuberculosis and drug resistance (DR), especially multi-drug resistant tuberculosis (MDR-TB), is a major public health problem in developing countries like Pakistan. The purpose of the current study was to see the frequency of drug resistance pattern of drug resistance tuberculosis (DR-TB) against 1st line anti-TB drugs. Drug susceptibility testing (DST) was performed for 473confirmed cultured positive isolates using proportion methods. The study period was started from November 2011 to June 2013 at a programmatic management of drug-resistant TB (PMDT) unit, Gulab Devi Chest Hospital Lahore, Pakistan. Out of the total, 291 (61.5%) patients were resistant to at least one anti-TB drugs and 246 (52%) were MDR-TB. Among 291 resistant patients, almost one-third of the patients were resistant to all the first-line drugs (n=113, 38.8%). The other prevalent resistance was found against rifampicin (n=277, 95.2%) and isoniazid (n=255, 87.6%) followed by ethambutol (n=181, 62.2%), streptomycin (n=176, 60.5%) and pyrazinamide (n=175, 60.1%). The proportion of males was relatively higher (n=151, 51.9%) than females, while previously treated patients were 175 (60.1%). Most of the cases were from age group 15-45 (81.1%) years, and belonged to the urban area (88.3%). Our study revealed high prevalence of drug-resistant TB, especially in the previously treated patients and young age peoples. Continuous monitoring of drug-resistant pattern of MDR-TB and their treatment is an essential requirement for future control in developing countries like Pakistan.

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## Introduction

Tuberculosis is an infectious and transferable disease to other peoples that is caused by *Mycobacterium* tuberculosis (MTB) (WHO 2014). One-third population of the world is considered to be influenced by tuberculosis (WHO, 2014). According to the world health organization (WHO), 9.6 million active TB cases ultimately has led to 1.5 million deaths in the year 2013. The rate of mortality in developing countries is greater than 95% of these 1.5 million deaths (WHO, 2014). Improper and adverse utilization of TB regimens can ultimately lead to multi drug resistant TB (MDR-TB). Resistance to at least two first line predominant anti-TB drugs i.e. isoniazid (INH) and rifampicin (RIF), is termed as MDR-TB (WHO, 2013a; Ullah *et al*., 2016a). MDR-TB is a hazard in reducing tuberculosis spread because of high cost treatment and difficult to treat the strains of MDR-TB(Diel *et al*., 2014; Nathanson *et al*., 2004).

Pakistan ranked5$^{th}$and 4$^{th}$ position among 22 high TB and 27 high MDR-TB burden countries, respectively(Ullah *et al*., 2016b). According to prevalence survey of TB, conducted in 2010-2011, the prevalence of tuberculosis cases is 341/100000 population in Pakistan(Ejaz Qadeer *et al*., 2016). MDR-TB in newly diagnosed TB is 4.3%, while in previously treated patients is 19.4%(Javaid, 2015). In selected population of Punjab, 19.4% MDR-TB was reported in retreated cases, while 4% in newly diagnosed patients(Ullah *et al*., 2016a). MDR-TB is present in 17.4% in close contact of MDR-TB patients(Javaid *et al*., 2016), in 3% population of new cases and 26% in retreated cases in selected population of Khyber Pakhtunkhwa (Ullah *et al*., 2016b).

In Pakistan, despite of several programs, majority of patients remain undiagnosed and untreated because case detection rate are quite low (63%) (Ullah *et al*., 2017). This low case detection is a challenge for National TB Control program. Increasing of case detection is very crucial for decreasing of further prevalence and transmission of TB and MDR-TB. The objective of the current study was to determine resistance pattern against first-line drugs in multi drug-resistant TB patients in Gulab Devi Chest Hospital (GDH) Lahore. The city of Lahore is the provincial metropolitan with a population of 12 million peoples, which came from all area of Punjab. Its shanty towns have a very high incidence of tuberculosis due to congested living style. Healthy people are obliged to live with tuberculosis patients which ultimately results in spread of tuberculosis. It is alarming to note that the patients of MDR-TB are transmitting the bacilli to healthy people which are difficult to treat.

## Materials and methods

*Study Setting*

The present study was carried out at the PMDT unit of GDH, Lahore, Pakistan. It is the biggest unit in the Punjab province, covering more than 32 TB endemic districts in this region.

*Study population*

A total of 507 cultured positive samples were enrolled from November 2011 to June 2013 in the present study. All suspected DR-TB patients, irrespective of sex and age were included in this study. *Mycobacterium* other than tuberculosis (MOTT), DST sensitive or those isolates on which DST could not applied were excluded from the analysis (Figure 1).

*Data collection procedure*

A special proforma was used for data collection by taking interview and reviewing medical record of the participants. The form included information associated to socio demographic, clinical and microbiological data of the patients. Information was collected on other variables including sex (male/female), age, area (urban/rural), family history of TB, previous history of anti-TB drugs treatment and economics status/occupation of the patients.

Previously treated patients were those who had previous history of anti-TB treatment, whereas newly diagnosed patients were those who did not have any anti-TB drugs treatment history or patients taken anti-TB drugs but less than one month.

*Decontamination and processing for culture*

One sample was collected from each patient and processed for smear microscopy, culture and DST. Concentrated method N-acetyl-L-cysteine sodium hydroxide (NALC-NaOH) was used for decontamination and culture processing. Decontaminated specimens were centrifuged at 3000xg for 15 minutes and sediments were used for culture and one drop for smear preparation(Aparna and Gokhale 2006; Ullah *et al*., 2014). After centrifugation, 0.2ml sediment was used for inoculation on Lowenstein Jensen (LJ) medium slant and incubated at 37°C up to 8 weeks or till growth. 0.5ml specimens was used for MGIT vials and incubated at 37°C after supplementation of PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocilin) to medium. Growth index were examined after 8 weeks or when there was positive growth.

Identification of *Mycobacterium tuberculosis*

Identification of MTB positive culture of LJ and MGIT vial were stained using Kinyoun and through differentiation test of BACTEC NAP TB (Becton Dickinson, USA). Nitrate reduction and Niacin positivity test were also used for differentiation of MTB and MOTT (Hasan *et al*., 2009; Khan *et al*., 2015).

*Drug susceptibility testing (DST) of Mycobacterium tuberculosis*

After confirmation of culture for MTB, agar proportion method was used for DST. Middle brook 7H10 medium (BBL) were used comprising concentrations of RIF (40µg/ml), INH (0.2µg/ml), streptomycin (SM) (4µg/ml) and ethambutol (EMB) (2µg/ml). For Pyrazinamide, we used BACTEC 7H12 medium with pH 6 at concentration of 100 µg/ml (BACTEC-TM PZA test medium, Becton Dickinson, USA). In each batch of DST, MTB H37Rv was use as control which is characteristically susceptible to all anti-TB drugs. Statistical analysis was performed using SPSS version 18 (SPSS Inc., Chicago, IL, USA). Categorical variables were summarized using frequencies and percentages whereas numerical variables were summarized with mean and standard deviation.

*Ethical approval*

The present research work was approved by the ethical committee of University of the Punjab, Lahore, Pakistan in accordance with the ethical standards of the responsible committee on human experimentation and with the latest (2008) version of Helsinki Declaration of 1975 (World Medical Association (WMA) 2009). The purpose of the study was explained and written consents from the patients or guardians were taken from all patients or from next of their kin, caretakers, or guardians/parents on behalf of all child participants.

**Results**

Out of total 473 patients, 291 (61.5%) patients were resistant to at least one drug included in the final study. Out of total drug resistant patients, 246 (52%) were MDR-TB and 113 (38.8%) showed resistance to all first line drugs. The proportion of males were relatively higher (n=151, 51.9%) than females.

**Table 1.** Patient's socio demographic characteristic.

| Variables | Frequency | Percentage |
|---|---|---|
| Gender | | |
| Male | 151 | 51.9 |
| Female | 140 | 48.1 |
| Marital status | | |
| Unmarried | 88 | 30.3 |
| Married | 203 | 69.7 |
| Age | | |
| Less than 15 | 10 | 3.4 |
| 15-45 years | 236 | 81.1 |
| 46-60 years | 36 | 12.4 |
| Above 60 years | 9 | 3.1 |
| Employment status | | |
| Employed | 88 | 30.3 |
| Unemployed | 97 | 33.3 |
| Student | 106 | 36.4 |
| Residence | 32 | 12.7 |
| Urban | | |
| Rural | 257 | 88.3 |

The mean age of patients was 32.45 (SD=14.93) years ranging from 10 to 92 years, and majority of the peoples (n=257, 88.3%) were belonged tour ban area.

Almost half of the patients (49%) were from the city of Lahore, 16% of the patients came from Kasur, 12% from Faisalabad and 5% from Toba Take Singh, and rest of them belonged to other parts of the Province of Punjab.

**Table 2.** Resistance pattern of *Mycobacterium tuberculosis* for first line drugs.

| Resistance pattern | Frequency | Percentage |
|---|---|---|
| Any resistance | | |
| R | 277 | 95.2 |
| I | 255 | 87.6 |
| Z | 175 | 60.1 |
| S | 176 | 60.5 |
| E | 181 | 62.2 |
| Mono resistance | | |
| R | 33 | 11.3 |
| I | 3 | 1 |
| Z | 1 | 0.3 |
| S | 1 | 0.3 |
| E | | |
| Poly resistance but non MDR | | |
| I + other drugs (except R) | 6 | 2.1 |
| R + other drugs (except) | 1 | 0.3 |
| Multi drug resistance | | |
| I + R only | 26 | 8.9 |
| I+ R + E only | 7 | 2.4 |
| I + R + S only | 14 | 4.8 |
| I + R + Z only | 16 | 5.5 |
| I + R + E + S | 28 | 9.6 |
| I + R + Z + S | 14 | 4.8 |
| I + R + E + Z | 28 | 9.6 |
| I + R + E + S + Z | 113 | 38.8 |

R= Rifampicin; I= Isoniazid; S= Streptomycin; E= Ethambutol; Z= Pyrazinamide

Other socio demographic characteristic details are present in Table 1. Previously treated patients were 175 (60.1%) while 116 (39.9%) patients had reported no history of treated with anti-TB drugs. Out of 175, 65 (37.1%) had completed the treatment course, in which 53 (30.3%) were cured, 50 (28.6%) treatment went failure and 7persons (4%) expired. None of the patient was HIV positive for infection after screening. Sputum smear test, at the start of treatment was positive for 170 (58.4%) and negative for 121(41.6%) samples.

The most commonly resistant combination of first line drugs was RIF+INH+ EMB+SM+PZA (n=113, 38.8%). The resistance for rifampicin (RIF) (n=277, 95.2%) was highest, either individual or in combination with other first line drugs (FLD) followed by isoniazid (n=255, 87.6%), ethambutol (n=181, 62.2%), streptomycin (n=176, 60.5%) and pyrazinamide (n=175, 60.1%). Details of the resistance pattern for FLD are presented in Table 2.

**Discussion**

Herein the current study, we presented resistance pattern of DR-TB in highly endemic area of Punjab, Pakistan. In the present study, 61.5% were resistant to at least one drug, 52% MDR-TB and 38%were resistant to all first line drugs which is in line with Irfan *et al* reported (64% resistant to at least one anti-TB drugs and 35.2% resistant to all first line drugs)(Seema*et al.*, 2006). However, TB resistance has also been reported to be different from different area.

The prevalence of drug resistance from 28-60%is present in different area of Pakistan(Butt *et al.*, 2004; Akhtar *et al.*, 2007; Alina Amjad *et al.*, 2012; Rao and Irfan, 2010).MDR-TB in Azerbaujan was 55.8%, 60% in Uzbekistan, whereas in India it is 17.2%(WHO, 2013b).

Our finding in this study about MDR-TB and resistance to all first line drugs are high than other studies like Karamat *et al.* (1999) who reported 14% MDR-TB, 21% to any drugs and 7% resistance to all first line drugs in Rawalpindi district of Punjab and Federal area of Islamabad in Pakistan(Karamat and Rafi, 1999). Butt *et al.* reported 28% MDR-TB and 7 % first line drug resistant(Butt *et al.*, 2004). The resistance in our study was very higher compared to above results; the probable reason may be we processed those specimens that were highly susceptible to DR-TB.

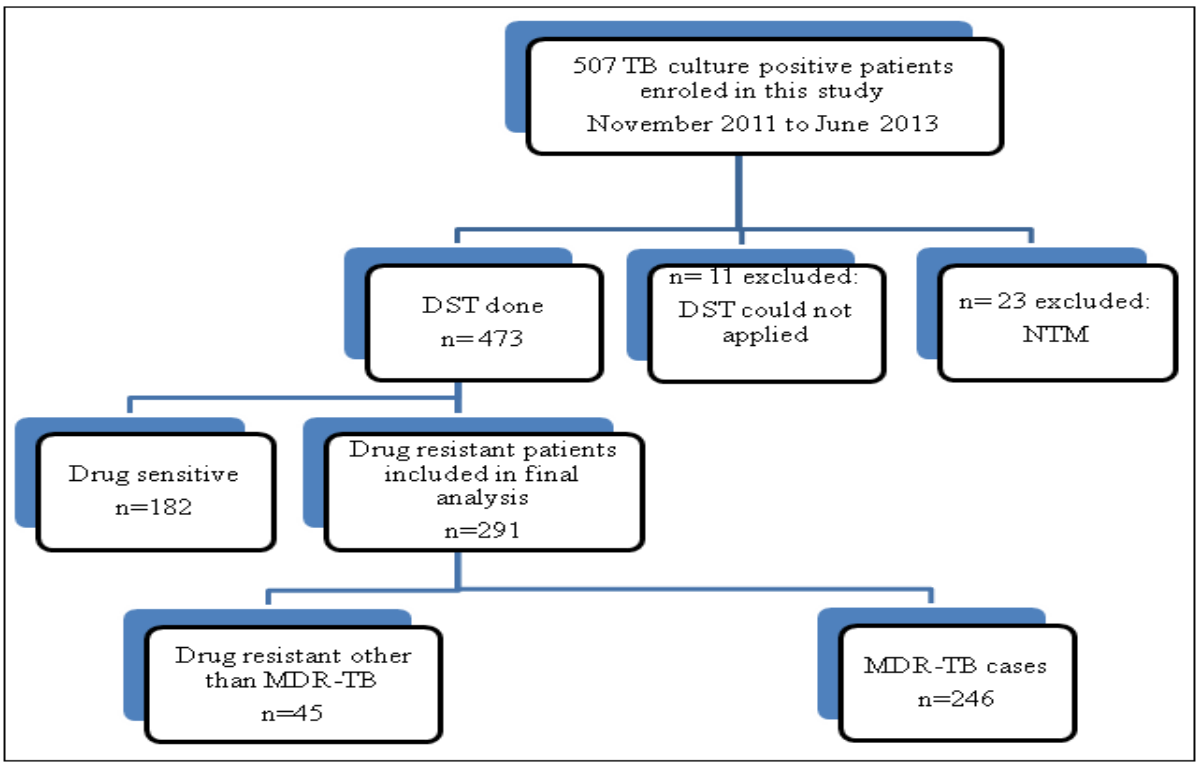


**Fig. 1.** Flow chart of enrolment, inclusion and exclusion of the study patients.

In this study, 60.1% drug resistance TB was present in those patients who had previous history of anti-TB drugs. Previous studies has shown that DR-TB has direct association with history of TB treatment(Seema Irfan *et al.*, 2006).Ullah *et al.*,(2016a) and Ruddy *et al.* (2015) reported that the prevalence of DR-TB is few times more in previously treated patients than those of newer treated (Ullah *et al.*, 2016a; Ruddy *et al.*, 2005). However, MDR-TB in newly diagnosed and previously treated patient shave also been reported to be different in different location of the world. In Japan, it is 9.8% in previously treated and 0.7% in newly diagnosed patients (Chacon *et al.*, 2009). In China, the reports showed frequency of 9.7% and 34.3% in new and previously treated patients respectively (Li *et al.*,2012). Micheletti *et al* reported that MDR-TB was 2.2% in newly diagnosed, 12% in previously treated and overall 4.7% in Brazil (Micheletti *et al.*, 2014). These dissimilarities in different area may be due to different levels of health care delivery system in different countries, socio-economic factor, living standards of peoples and TB control program. The high resistance in previous treated patients' cases is sign of lack of treatment supervision, poor compliance and ineffective TB Control Programme, while new cases may be due to transmission of resistant TB to healthy peoples. The effective TB Control programs lead to decrease in DR-TB pattern. Complete and adequate treatment for tuberculosis patients should be confirmed for avoiding spread of and decrease in resistance of TB, which is big a challenge and significant threat to National TB Control Program (Javaid, 2015; Ullah*et al.*, 2016a).

In the present study, 81.1% of drug resistant were present in the peoples from age group between 15-45 years. In the literature studies, different peoples used different cut-off points of age group, so there is no well-established link between MDR-TB and age group. However, the high resistant TB in this age group may be probable reason, as the peoples are often busy in different activities like work, studies, or other activities on day-to-day basis as compared to other inactive lifestyle peoples of older age. In the current study, all patients were belonged to low socioeconomic status which is line with other studies conducted in Pakistan(Rao and Irfan 2010).

The limitation of the study was low number and very highly selected drug resistant suspected patients so the results cannot be generalized on general populations. Secondly, we were unable to present results of second line drugs so the prevalence of XDR-TB may be underestimated.

## Conclusion

A very high rate of MDR-TB and resistance against all first line drugs was found in the present study. Most of the cases were present in working age peoples and in previously treated patients. The current statistic highlights the need of properly monitoring of patients confirming obedience and completion of treatment and regular surveillance of drug resistance TB

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## References

**Akhtar S, Haidri FR, Memon AM.** 2007. "Original Article Drug Resistance to Tuberculosis in a Tertiary Care Setting in Karachi" The Journal of the Pakistan Medical Association. **57(6),** 282–84.PMID: 17629227.

**Amjad A, Satti L, Farwa U, Abbasi SA.** 2012. "Rising Trend of Multi Drug Resistant Tuberculosis : A Threat to Community" **10(1),** 32–36.

**Aparna S, Moorthy KVK, Gokhale S.** 2006. "From Microscopy Centre to Culture Laboratory: A Viable Ride for Mycobacteria." International Journal of Tuberculosis and Lung Disease **10(4),** 447–49. PMID: 16602411.

**Butt T, Ahmad RN, Kazmi SY, Rafi N.** 2004. "Multi-Drug Resistant Tuberculosis in Northern Pakistan." The Journal of the Pakistan Medical Association **54,** 469–72.PMID: 15518370.

**Chacon L, Lainez M, Rosales E, Mercado, Caminero JA**. 2009. "Evolution in the Resistance of Mycobacterium Tuberculosis to Anti-Tuberculosis Drugs in Nicaragua." The International Journal of Tuberculosis and Lung Disease : The Official Journal of the International Union against Tuberculosis and Lung Disease **13(1),** 62–67.PMID: 19105880

**Diel R, Vandeputte J, de Vries G, Stillo J, Wanlin M, Nienhaus A.** 2014. "Costs of Tuberculosis Disease in the European Union : A Systematic Analysis and Cost Calculation," 554–65. http://dx.doi.org/10.1183/09031936.00079413.

**Qadeer E, Fatima R, Yaqoob A, Tahseen S, Haq MU, Ghafoor A, Asif M, Straetemans M, Tiemersma EW.** 2016. "Population Based National Tuberculosis Prevalence Survey among Adults (>15 Years) in Pakistan, 2010–2011." PLoS ONE 1 **11(2),** e0148293. http://dx.doi.org/10.1371/journal. pone.0148293.

**Hasan R, Jabeen K, Mehraj V, Zafar F, Malik F, Hassan Q, Azam I, Kadir MM.** 2009. "Trends in Mycobacterium Tuberculosis Resistance, Pakistan, 1990-2007." International Journal of Infectious Diseases **13(6)**. http://dx.doi.org/10.1016/j.ijid.2009.01.008.

**Javaid, Arshad.** 2015. "Burden of Mdr-Tb and Its its Control in Pakistan." Pakistan Journal Chest Medicine, No. January **21(04),** 131–33.

**Javaid A, Khan MA, Khan MA, Mehreen S, Basit A, Khan RA, Ihtesham M, Ullah I, Khan A, Ullah U.** 2016. "Screening Outcomes of Household Contacts of Multidrug-Resistant Tuberculosis Patients in Peshawar, Pakistan." Asian Pacific Journal of Tropical Medicine **9(9),** Elsevier B.V.: 909–12. http://dx.doi.org/10.1016/j.apjtm.2016.07.017.

**Karamat KA, Shahid Rafi.** 1999. "Drug Resistance in Mycobacterium Tuberculosis : A Four Years Experience." The Journal of the Pakistan Medical Association, **49,** 262–65. PMID: 10647236.

**Khan MA, Mehreen S, Basit A, Khan RA, Jan F, Ullah I, Ihtesham M, Khan A, Ullah U, Javaid A.** 2015. "Characteristics and Treatment Outcomes of Patients with Multi-Drug Resistant Tuberculosis at a Tertiary Care Hospital in Peshawar, Pakistan." Saudi Medical Journal **36(12),** 1463–71, http://dx.doi.org/10.15537/smj.2015.12.12155.

**Micheletti VCD, Moreira JDS, Ribeiro MO, Kritski AL, Braga JU.** 2014. "Drug-Resistant Tuberculosis in Subjects Included in the Second National Survey on Antituberculosis Drug Resistance in Porto Alegre, Brazil." J Brasileiro de Pneumologia **40(2),** 155–63.
PMID: 24831400 PMCID: PMC4083645

**Nathanson E, Gupta R, Huamani P, Leimane V, Pasechnikov AD, Tupasi TE, Vink K, Jaramillo E, Espinal MA.** 2004. "Adverse Events in the Treatment of Multidrug-Resistant Tuberculosis: Results from the DOTS-Plus Initiative." International Journal of Tuberculosis and Lung Disease **8(11),** 1382–84.PMID: 15581210

**Rao NA, Irfan M, Soomro MM, Mehfooz Z.** 2010. Drug resistance pattern in multidrug resistance pulmonary tuberculosis patients. Journal of the College of Physicians and Surgeons Pakistan. **20(4),** 262-265.

**Ruddy M, Balabanova Y, Graham C, Fedorin I, Malomanova N, Elisarova E, Kuznetznov S, Gusarova G, Zakharova S, Melentyev A, Krukova E.** 2005. "Rates of Drug Resistance and Risk Factor Analysis in Civilian and Prison Patients with Tuberculosis in Samara Region, Russia," **60(2),** 130–36.
http://dx.doi.org/10.1136/thx.2004.026922.

**Seema I, Hassan Q, Hasan R.** Assessment of resistance in multi drug resistant tuberculosis patients. JOURNAL-PAKISTAN MEDICAL ASSOCIATION. 2006 Sep 1, **56(9),** 397-400.PMID: 17091751

**Ullah I, Javaid A, Masud H, Ali M, Basit A, Ahmad W, Younis F, Yasmin R, Khan A, Jabbar A, Husain M.** Rapid detection of Mycobacterium tuberculosis and rifampicin resistance in extrapulmonary tuberculosis and sputum smear-negative pulmonary suspects using Xpert MTB/RIF. Journal of Medical Microbiology. 2017 Apr 28, **66(4),** 412-418.
http://dx.doi.org/10.1099/jmm.0.000449.

**Ullah I, Javaid A, Tahir Z, Ullah O, Shah AA, Hasan F, Ayub N.** 2016a. "Pattern of Drug Resistance and Risk Factors Associated with Development of Drug Resistant Mycobacterium Tuberculosis in,"**11(1),** 1–7,
http://dx.doi.org/10.1371/journal.pone.0147529.

**Ullah I, Shah AA, Basit A, Ali M, Ullah U, Ihtesham M, Mehreen S, Mughal A, Javaid A.** 2016b. "Rifampicin Resistance Mutations in the 81 Bp RRDR of Rpo B Gene in Mycobacterium Tuberculosis Clinical Isolates Using Xpert MTB / RIF in Khyber Pakhtunkhwa , Pakistan : A Retrospective Study." *BMC Infectious Diseases*. BMC Infectious Diseases, **16(1),** 4–9,
http://dx.doi.org/10.1186/s12879–016–1745–2.

**Ullah I, Shah AA, Tahir Z, Hasan F, Ayub N**. 2014. "Detection of Mycobacterium Tuberculosis from Clinical Specimens by Conventional and Molecular Technique in Punjab , Pakistan." Global Veterinaria **13(6),** 1002–9,
http://dx.doi.org/10.5829/idosi.gv.2014.13.06.9158.

**WHO.** 2013a. "Global Tuberculosis Report 2013." World Health Organization, 306.
http://dx.doi.org/10.3917/spub.092.0139. 2013b.

**WHO.** Multidrug-Resistant Tuberculosis in Belarus: The Size of the Problem and Associated Risk Factors." WHO. World Health Organization. 2014. "Global Tuberculosis Report 2014." *WHO Report* 2014 **(4),** 171.
http://dx.doi.org/10.1155/2014/187842.

**World Medical Association (WMA).** 2009. "Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects." Jahrbuch Für Wissenschaft Und Ethik **14(1).** http://dx.doi.org/10.1515/9783110208856.233.

**Li X, Wang H, Jing H, Wang Y, Yu C, Wang J, Liu Z, Graviss EA, Ma X.** 2012. "Population-Based Surveillance of Extensively Drug-Resistant Tuberculosis in Shandong," no. March 2012, **16,** 612–14. http://dx.doi.org/10.5588/ijtld.11.0507.