Original Article

The occurrence of multidrug-resistant *Mycobacterium tuberculosis* from patients of pulmonary tuberculosis

Azhar Iqbal[1], Muhammad Shafique[1], Muhammad Asif Zahoor[1], Saima Muzammil[1], Zeeshan Nawaz[1], Abdul Jabbar[2], Mohsin Khurshid[1], Riaz Hussain[3], Md. Akhtarul Islam[4], Ahmad Almatroudi[5], Khaled Saleh Allemailem[5], Muhammad Hidayat Rasool[1], Bilal Aslam[1]

[1] Department of Microbiology, Government College University Faisalabad, Pakistan
[2] Department of Clinical Medicine, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan
[3] University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan
[4] Statistics Discipline, Science, Engineering and Technology School, Khulna University Khulna, Bangladesh
[5] Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraydah, Saudi Arabia

Abstract
Introduction: Multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) is one of the leading causes of death in the world. The resource constraints make it difficult to diagnose and monitor the cases of MDR-TB. GeneXpert is a recognized tool used to diagnose the patients of pulmonary tuberculosis in clinical settings across the globe.
Methodology: The present one-year cross-sectional study was conducted to estimate the occurrence of MDR-TB in patients with pulmonary TB. A total of 1000 patients suspected of pulmonary tuberculosis were included in this study. A random convenient sampling technique was done to collect the sputum samples (twice) from the patients. Samples were processed for the detection of *Mycobacterium tuberculosis* using conventional detection methods like the Ziehl Nelson staining method and fluorescent microscopy. Additionally, Cepheid GeneXpert was used for molecular detection of MDR-TB in smear-positive samples of pulmonary tuberculosis by amplifying the rifampicin resistance determining region (RRDR; *rpoB* gene). All the tests were performed in the biosafety level III lab of District Headquarters Hospital Nankana Sahib.
Results: It was observed that 103 (10.3%) individuals were diagnosed as positive for tuberculosis among 1000 patients. Among these 103 TB positive cases, there were 11 (10.7%) patients diagnosed with rifampicin resistance gene (RR-Gene) of *Mycobacterium tuberculosis*.
Conclusions: Overall findings of the study showed that MDR-TB is prevalent in pulmonary TB patients and GeneXpert is the most sensitive technique for early diagnosis of the disease, which may be very helpful in the treatment and control of this public health menace in low and middle-income countries.

Key words: Tuberculosis; MDR; TB; rifampicin; fluorescent microscopy; Pakistan.

Introduction

Tuberculosis, caused by *Mycobacterium tuberculosis* (MTB), is one of the most common diseases affecting humans worldwide [1]. Multidrug-resistant tuberculosis (MDR-TB) is a well-recognized global health threat, that emerged due to the development of resistance against Isoniazid (INH) and Rifampicin (RIF), the two best known first-line anti-tuberculosis drugs. It has been reported that up to 20% of TB cases across the globe are MDR-TB [2]. In Asia, Central Asia has the highest prevalence of MDR-TB; while in Europe, Eastern Europe has more cases of MDR-TB as compared to the other regions.

Disturbingly, about 60% prevalence rate of MDR-TB has been reported from India, China, and Russia [2]. In addition to that extensively drug-resistant-tuberculosis (XDR-TB), which showed resistance to quinolones as well has also been reported from various parts of the world [3]. Prompt and precise diagnosis of MDR-TB is very critical for the management of the infection; it also restrains the dissemination of disease [3-5]. Mutation in *rpoB* gene resided at rifampicin resistance determining region (RRDR) is associated with the resistance in MDR-TB against first-line drugs [6], the mutation in cluster II region or N-terminal of the *rpoB* gene may also be a factor involved in the development of

resistance in MDR-TB [4,6]. Development of antibiotic resistance leads to the treatment failure and emergence of MDR-TB [4,6].

Mutation detection of *rpoB* gene in MDR-TB through molecular assays has been well recognized and has a wide application in clinical diagnostic settings [4,5]. The Xpert MTB/RIF (Xpert) is one of the recommended assays by the World Health Organization (WHO) for the diagnosis of MDR-TB [5,7]. This assay is a DNA amplification-based test that detects DNA of the bacterium and RIF resistance simultaneously in a very short time. GeneXpert is a semi-automated instrument being used with GeneXpert(R) System (Cepheid, Sunnyvale, California, USA). It is a nested real-time PCR in-vitro diagnostic test for the detection of complex DNA of MTB and RIF resistance-associated mutations of *rpo*B in sputum samples from patients at risk for rifampicin resistance (RR) gene [8].

Pakistan is a low middle-income country and ranks 5th among nations with the highest TB burden across the globe. Pakistan is also listed among countries where MDR-TB is a major challenge in health care settings [9]. Key reasons associated with the high prevalence of MDR-TB in the country include illiteracy, population, poverty, and inadequate monitoring and control measures. Studies from Pakistan have shown the diagnostic accuracy of GeneXpert assay used to detect

the rifampicin resistance in clinical TB patients. In addition to that, Xpert MTB/RIF assay has also been evaluated to diagnose pulmonary TB in pediatric patients in local resource-limited health care settings [9]. So, the present study was designed to estimate the occurrence of MDR-TB in pulmonary patients and associated risk factors of the infection by using the GeneXpert(R) assay.

Methodology

Study settings

In this one-year cross-sectional study, samples were collected from patients who came to the pulmonology department of District Headquarters Hospital Nankana Sahib. All collected samples were transported to the Tuberculosis-Directly Observed Treatment, Short-course (TB-DOTS) lab of District Headquarters (DHQ) hospital for further processing. At the time of sample collection, a pre-designed questionnaire was administered to the guardians of patients to collect additional data related to the study. All the tests of the study were performed in the biosafety level III lab of DHQ hospital, Nankana Sahib, Punjab, Pakistan.

Inclusion and Exclusion Criteria

Only patients suspected of pulmonary tuberculosis based on their clinical presentation were included in the study. Both male and female patients were included in the study. They could be either be new cases or follow-up cases. Patients less than 12 years of age were excluded from the study.

Sample Collection

Sample collection was done by employing a convenient sampling technique. A total of (N = 1000) samples were collected; among them, 555 were female samples and 445 were male samples. Among the collected samples 814 were newly diagnosed cases while 186 were follow-up cases. A total of 653 patients were from urban areas and 347 patients were from the rural areas. These samples were divided into various age groups; details of the age group are given in Table 1. Moreover, various risk factors associated with the diseases like education, job, residence, contact history, and new or follow-up cases were also studied.

Processing of the Samples

All the patients included were provided with a clean, sterile, leak-proof wide-mouthed plastic container and requested for sample (sputum) collection.

Table 1. Basic information of the respondents.

Categories	n (%)
TB Status	
Yes	107 (10.7)
No	893 (89.3)
Type of Place of Residence	
Rural	653 (65.3)
Urban	347 (34.7)
Gender	
Male	555 (55.5)
Female	445 (44.5)
Education	
Not Educated	463 (46.3)
Educated	532 (53.2)
Occupation	
Not Working	694 (69.4)
Working	306 (30.6)
Age	
≤ 20	190 (19.0)
21-35	270 (27.0)
36-50	261 (26.1)
51-65	184 (18.4)
> 65	95 (9.5)
TB Contact	
No	750 (75.0)
Yes	250 (25.0)
Treatment	
New Case	814 (81.4)
Follow-up	186 (18.6)

Ziehl Nielsen Staining

Smears were prepared from sputum samples by using the wooden applicator. The conventional procedure was adopted for Ziehl Nielsen (ZN) staining method. Briefly, smears were covered with Carbol Fuchsin (Merck, Darmstadt, Germany) and provided heat till vapors begin to rise and left for 5 minutes, washed, and covered with 5% HCl followed by gentle washing. All the smears were air-dried and examined under the light microscope (Leica Microsystems™, Germany) under an oil immersion objective lens [10,11].

Auramine-O Staining and Florescent Microscopy

For Light Emitting Diode Fluorescence Microscopy (LED-FM) microscopy smears were covered with auramine-O and left for 10 minutes and rinsed with acid alcohol followed by gentle washing. Subsequently, smears were covered with methylene blue for 1 minute followed by gentle washing. All the smears were air-dried and examined under a 40X lens using Primo Star iLED (ZEISS™, Germany). Smears were examined for the presence or absence of Mycobacterium tuberculosis and reposted by following WHO/ International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines [12].

GeneXpert MTB/RIF assay

All sputum samples were shifted from container to master mix solution and left for 10 minutes. Then transferred to the cartridge and lidded into the Cepheid GeneXpert(R) System. The assay was performed according to the manufacturer protocol. Untreated sputum sample was mixed with sample reagent with a ratio of 1:2, followed by a vigorous manual mixing. The sample was incubated at room temperature for 15 minutes, following which it was shaken again, and kept aside for 10 minutes Total 2 mL of sample was dispensed into the cartridge and placed inside the GeneXpert(R) System (Cepheid, Sunnyvale, California, USA). Once the assay was complete, the results were recorded and interpreted using the inbuilt software.

Statistical analysis

Statistical software SPSS V.27 (SPSS Inc. Chicago, USA) was used to carry out the analysis. Univariate analysis, bivariate analysis (Chi-Square test), and multivariate analysis (binary logistic regression model) were used to determine the key factors that have an impact on TB status.

Results

The occurrence of Mycobacterium tuberculosis

Overall, 103 (10.3%) out of 1000 patients were diagnosed as positive for tuberculosis. These individuals were considered positive for

Table 2. Assessing the association between TB status and selected factors with *p* values obtained from the chi-square test.

Factors	TB status		*p* value
	No	Yes	
Type of Place of Residence			
Rural	591 (59.1)	62 (6.2)	0.091
Urban	302 (30.2)	45 (4.5)	
Gender			
Male	482 (48.2)	73 (7.3)	0.005
Female	411 (41.1)	34 (3.4)	
Education			
Not Educated	415 (41.5)	51 (5.1)	0.588
Educated	479 (47.9)	55 (5.5)	
Occupation			
No	632 (63.2)	62 (6.2)	0.007
Yes	261 (26.1)	45 (4.5)	
Age of the respondent			
≤ 20	172 (17.2)	18 (1.8)	
21-35	236 (23.6)	34 (3.4)	0.029
36-50	241 (24.1)	20 (2)	
51-65	160 (16)	24 (2.4)	
> 65	84 (8.4)	11 (1.1)	
TB Contact Status			
No	675 (67.5)	75 (7.5)	0.215
Yes	218 (21.8)	32 (3.2)	
Treatment			
New Case	719 (71.9)	95 (9.5)	0.038
Follow-up	174 (17.4)	12 (1.2)	

Mycobacterium tuberculosis based on GeneXpert results (Table 1).

Associated risk factors of Mycobacterium tuberculosis

Among positive patients, a total of 70 (12.6%) were males while 33 (7.4%) were females. Positive patients from urban and rural areas were 45 (43.7%) and 58 (56.3%), respectively. Patients with and without a history of contact with known TB positive cases that found positive for *Mycobacterium tuberculosis* were 72 (69.9%) and 31 (30.1%), respectively. Total 92 (89.3%) patients were diagnosed as first time infected with *Mycobacterium tuberculosis* while 11 (10.7%) patients were already on anti-tuberculosis therapy. Details of statistical analysis showing the association between TB status and selected factors are given in Table 2.

Occurrence of MDR-TB

Among 103 TB positive cases, total 11 (10.7%) patients were diagnosed with MDR-TB including 8 (72.7%) males and 3 (23.3%) female patients. The distribution of MDR-TB among urban and rural areas were 1 (9.1%) and 10 (90.9%), respectively. While patients with and without a history of contact with known TB positive cases have 1 (9.1%) and 10 (90.9%)

MDR-TB rate, respectively. Moreover, out of 103 positive samples total of 10 (90.9%) were found as MDR-TB in case of patients who were diagnosed for the first time with *Mycobacterium tuberculosis* while 1 (9.1%) patient who was already on anti-tuberculosis therapy was MDR-TB positive.

Comparison of techniques used in the study

Overall, out of 1000 collected samples, it was observed that the ZN staining technique is less sensitive as compared to the GeneXpert. In case of ZN staining, a total of 78 (7.8%) samples were found to be true positive sample, 3 (0.3%) samples were false-positives, and 22 (2.5%) samples were recorded as a false negative. In the case of auramine staining, 94 (9.4%) samples were found true positive and 90 (9.0%) were true negative. Whereas 1 (0.1%) sample was observed as false-positive, and 8 (0.8%) samples were recorded as a false negative.

Among the three techniques used in the study, as expected GeneXpert was found to be the most sensitive and specific technique. In the case of GeneXpert total of 103 (10.3%) samples were true positive and 897 (89.7%) samples were true negative, and none of the

Table 3. Logistic regression model based adjusted effects of the selected factor for TB status.

	Coefficient (B)	Odds Ratio (OR)	*p* value	95% C.I. for OR	
				Lower	Upper
Place of Residence					
Rural					
Urban	0.520	1.681	0.016	0.815	3.469
Education					
Not Educated					
Educated	-0.703	0.495	0.007	0.296	0.828
Occupation					
Unemployed					
Working	0.362	1.436	0.153	0.875	2.356
Age of the Respondent					
≤ 20					
21-35	-0.794	0.452	0.001	0.277	0.738
36-50	-1.527	0.217	0.000	0.121	0.390
51-65	-0.918	0.399	0.000	0.239	0.667
> 65	-0.845	0.430	0.020	0.211	0.875
Gender					
Male					
Female	-0.941	0.390	0.000	0.254	0.599
TB contact					
No					
Yes	-0.323	0.724	0.388	0.347	1.507
Treatment					
New Case					
Follow-up	-0.828	0.437	0.000	0.310	0.616
Model Summary					
-2 Log likelihood				705.934a	
Cox & Snell R Square				0.492	
Nagelkerke R Square				0.656	

samples were recorded as false positive or false negative.

The people living in urban regions are 1.681 (OR = 1.681, CI: 0.815, 3.469) times more likely to be affected by TB than people living in rural regions. The variable education illustrates that educated respondents are 0.495 (OR = 0.495, CI: 0.296, 0.828) times less likely to be affected by TB compared to the respondent who is not educated. Again, the age of the respondent was strongly associated with TB status and showed that respondents aged more than 20 years are less likely to be affected by TB than the respondent younger than 20 years. Male respondents were more likely to be affected by TB than the female respondents. Table 3 indicated that female respondent were 0.390 times (OR = 0.390, CI: 0.254, 0.599). A respondent with follow-up treatment was 0.437 (OR = 0.437, CI: 0.310, 0.616) times less likely to be affected by TB than a respondent with new case treatment. Form model summary we realized that this model could explain the 49% to 66% percent of the total variations (Table 3).

Discussion

The current study was designed to estimate the occurrence of MDR-TB in pulmonary patients, and findings revealed that 10.7% of pulmonary patients were infected with MDR-TB. The results of the present study are in line with several other studies from Pakistan (Ullah *et al.*, 2015). Results showed that MDR-TB is more prevalent in males as compared to females, other significant ($p < 0.05$) risk factors include rural areas.

Additionally, the occurrence of MDR-TB was significantly high ($p < 0.05$) in new patients who were diagnosed for the first time with *Mycobacterium tuberculosis*. The results of the study are in agreement with the study from Pakistan [1], however previous studies [13] have shown disagreement with these results. These studies found that patients who have been treated earlier treated have a higher prevalence of MDR-TB as compared to freshly diagnosed TB patients [14]. The increasing occurrence of MDR-TB is a significant threat to TB control programs in developing countries [15].

Results of the present study are also comparable with some studies from the neighboring country i.e., India. These studies reported a high occurrence rate of MDR-TB in TB-treated patients [16]. Similar results were reported in a global project conducted by World Health Organization (WHO) in India, they reported about 17% prevalence of MDR-TB in patients previously treated with anti-TB drugs [2].

The occurrence of RR-TB in the study population is a severe concern for the health authorities. The resistance rate reported in the study is similar to previously reported data from Pakistan [12,17,18]. Additionally, a similar prevalence rate of RR-TB was reported from other regions of the world like Ethiopia and Nigeria, 10.3% and 12%, respectively [19-21]. Some studies have reported variable occurrence of RR-TB, this variation in the prevalence of RR-TB may be due to factors like study settings, sample size, patient selection, and environmental factors. Findings of the study revealed that male patients had a higher rate of RR-TB as compared to female patients, results of the present study are comparable with the data reported from Sindh province in Pakistan), that shows a high incidence rate of MDR-TB in male (53%) as compared to females 47% [18]. Particularly in Pakistan, inadequate diagnostic and therapeutic facilities in association with other socioeconomic crises play a major role in the emergence of MDR-TB [17,22]. A range of data has been reported from Pakistan showing an increasing incidence rate of MDR-TB as well as extensively drug-resistant TB (XDR-TB). It has been reported that the incidence rate of MDR-TB has jumped from 5% to almost 34% [23], another local study has reported an incidence rate of 5% for MDR-TB in female patients [24]. A study from Iran, a neighboring country of Pakistan, has also reported similar trends in the occurrence of MDR-TB with a 15% prevalence rate in their study population [25].

Results of the study revealed that patients from the rural area have a high incidence rate (90 %) of MDR-TB as compared to the urban patients. Findings are in accordance with previously reported studies from the country [23], lack of awareness campaign in a rural area, restricted living pattern, and poverty may contribute substantially to the prevalence rate of MDR-TB in rural areas of Pakistan. In contrast to the study by Ullah *et al.* [1] that reported high incidence in follow-up cases, findings of the present study showed a high rate (90%) of MDR-TB in new cases as compared to follow-up cases. These contrary results may be due to the variation of the study settings and study population of these investigations. Overall, results of the study showed that various risk factors like residence ($p < 0.091$), gender ($p < 0.005$), age ($p < 0.029$), occupation ($p < 0.007$) and treatment ($p < 0.038$) were significantly associated with the incidence of MDR-TB (Table 2).

Moreover, in the present study, we also assessed the diagnostic yield and sensitivity of GeneXpert to identify MDR-TB in sputum samples compared with two conventional techniques, ZN staining and auramine

staining, considered as the gold standard in TB diagnostics. Several studies have reported the diagnostic efficacy of GeneXpert in the case of pulmonary TB [26-28]. In the present study, it was found that GeneXpert is the most sensitive and specific technique for TB diagnosis, as compared to the conventional and gold standard techniques. In local clinical settings the use of GeneXpert for diagnosing MDR-TB in clinical patients has been well-documented. Similar to the findings to the present study other studies from Pakistan have also reported the diagnostic accuracy of GeneXpert, which revealed that it is significantly sensitive as well as specific tool for MDR-TB diagnosis as compared to other conventional diagnostic approaches [29,30].

Results of the study carry substantial significance because of limited data regarding the occurrence of MDR-TB in pulmonary patients. Since antibiotic resistance is an evolving mechanism, there is a need for continuous monitoring of MDR-TB strains. Some limitations of the study include bias in patient selection since the study was based at a TB center, results of the study may not be useful for overall TB status in the region as samples were collected from pulmonary patients and a strict exclusion criterion was adopted for the collection of samples which may be differ from the actual field situation.

In conclusion, MDR-TB is a persistently evolving health concern. Efficient diagnostic procedures like GeneXpert may be very useful to detect MDR-TB cases, which may be beneficial to manage the infection. Additionally, associated risk factors should be considered to develop a practical preventive strategy against this public health menace.

References

1. Ullah I, Javaid A, Tahir Z, Ullah O, Shah AA, Hasan F, Ayub N (2016) Pattern of drug resistance and risk factors associated with development of drug resistant *Mycobacterium tuberculosis* in Pakistan. PLoS One 1: e0147529.
2. World Health Organization (2013) Global tuberculosis report. Available: https://apps.who.int/iris/handle/10665/91355. Accessed: 02/03/2020.
3. Seung KJ, Keshavjee S, Rich ML (2015) Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Cold Spring Harb Perspect Med 5: a017863.
4. Ahmad S, Mokaddas E (2014) Current status and future trends in the diagnosis and treatment of drug-susceptible and multidrug-resistant tuberculosis. J Infect Public Health 7: 75-91.
5. Drobniewski F, Nikolayevskyy V, Balabanova Y, Bang D, Papaventsis D (2012) Diagnosis of tuberculosis and drug resistance: what can new tools bring us? Int J Tuberc Lung Dis 16: 860-870.
6. Telenti A, Imboden P, Marchesi F, Matter L, Schopfer K, Bodmer T, Lowrie D, Colston M, Cole S (1993) Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet 341: 647-651.
7. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD (2010) Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363: 1005-1015.
8. Churchyard GJ, Stevens WS, Mametja LD, McCarthy KM, Chihota V, Nicol MP, Erasmus LK, Ndjeka NO, Mvusi L, Vassall A (2015) Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: A cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF. Lancet Glob Health 3: e450-e457.
9. Iqbal F, Defer MK, Latif A, Hadi H (2020) Understanding how geographic, demographic and treatment history impact health outcomes of patients with multi-drug-resistant tuberculosis in Pakistan, 2014-2017. Epidemiol Infect 148: e253.
10. Armbruster C (1994) Infections with *Mycobacterium tuberculosis* and MOTT (*Mycobacteria* other than tuberculosis) in HIV infected and AIDS patients. Wien Med Wochenschr 144: 182-186.
11. Chen P, Shi M, Feng GD, Liu JY, Wang BJ, Shi XD, Ma L, Liu XD, Yang YN, Dai W, Liu TT, He Y, Li JG, Hao XK, Zhao G (2012) A highly efficient Ziehl-Neelsen stain: Identifying de novo intracellular *Mycobacterium tuberculosis* and improving detection of extracellular *M. tuberculosis* in cerebrospinal fluid. J Clin Microbiol 50: 1166-1170.
12. Khan AS, Ali S, Khan MT, Ahmed S, Khattak Y, Irfan M, Sajjad W (2018) Comparison of GeneXpert MTB/RIF assay and LED-FM microscopy for the diagnosis of extra pulmonary tuberculosis in Khyber Pakhtunkhwa, Pakistan. Braz J Microbiol 49: 909-913.
13. den Boon S, van Lill SW, Borgdorff MW, Enarson DA, Verver S, Bateman ED, Irusen E, Lombard CJ, White NW, de Villiers C, Beyers N (2007) High prevalence of tuberculosis in previously treated patients, Cape Town, South Africa. Emerg Infect Dis 13: 1189-1194.
14. Ruddy M, Balabanova Y, Graham C, Fedorin I, Malomanova N, Elisarova E, Kuznetznov S, Gusarova G, Zakharova S, Melentyev A, Krukova E, Golishevskaya V, Erokhin V, Dorozhkova I, Drobniewski F (2005) Rates of drug resistance

and risk factor analysis in civilian and prison patients with tuberculosis in Samara Region, Russia. Thorax 60: 130-135.

15. Hashmi HJ, Javed H, Jamil N (2017) Emerging epidemic of drug resistant tuberculosis in vulnerable populations of developing countries. Afr Health Sci 17: 599-602.

16. Sharma SK, Kaushik G, Jha B, George N, Arora SK, Gupta D, Singh U, Hanif M, Vashisht RP (2011) Prevalence of multidrug-resistant tuberculosis among newly diagnosed cases of sputum-positive pulmonary tuberculosis. Indian J Med Res 133: 308-311.

17. Khan A, Walley J, Newell J, Imdad N (2000) Tuberculosis in Pakistan: Socio-cultural constraints and opportunities in treatment. Soc Sci Med 50: 247-254.

18. Khan RA, Shaikh AA, Bulaadi GQ (2019) Incidence of multidrug-resistant tuberculosis in Sindh, Pakistan. Cureus 11: e4571.

19. Arega B, Menbere F, Getachew Y (2019) Prevalence of rifampicin resistant *Mycobacterium tuberculosis* among presumptive tuberculosis patients in selected governmental hospitals in Addis Ababa, Ethiopia. BMC Infect Dis 19: 307.

20. Geleta DA, Megerssa YC, Gudeta AN, Akalu GT, Debele MT, Tulu KD (2015) Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in sputum specimens in remote health care facility. BMC Microbiol 15: 220.

21. Mulu W, Abera B, Yimer M, Hailu T, Ayele H, Abate D (2017) Rifampicin-resistance pattern of *Mycobacterium tuberculosis* and associated factors among presumptive tuberculosis patients referred to Debre Markos Referral Hospital, Ethiopia: A cross-sectional study. BMC Res Notes 10: 8.

22. Thomas BE, Shanmugam P, Malaisamy M, Ovung S, Suresh C, Subbaraman R, Adinarayanan S, Nagarajan K (2016) Psycho-socio-economic issues challenging multidrug resistant tuberculosis patients: A systematic review. PLoS One 11: e0147397.

23. Hasan R, Jabeen K, Ali A, Rafiq Y, Laiq R, Malik B, Tanveer M, Groenheit R, Ghebremichael S, Hoffner S, Hasan Z (2010) Extensively drug-resistant tuberculosis, Pakistan. Emerg Infect Dis 16: 1473-1475.

24. Ejaz M, Siddiqui AR, Rafiq Y, Malik F, Channa A, Mangi R, Habib F, Hasan R (2010) Prevalence of multi-drug resistant tuberculosis in Karachi, Pakistan: Identification of at-risk groups. Trans R Soc Trop Med Hyg 104: 511-517.

25. Wahab F, Ashraf S, Khan N, Anwar R, Afridi MZ (2009) Risk factors for multi-drug resistant tuberculosis in patients at tertiary care hospital, Peshawar. J Coll Physicians Surg Pak 19: 162-164.

26. Bowles EC, Freyée B, van Ingen J, Mulder B, Boeree MJ, van Soolingen D (2011) Xpert MTB/RIF®, a novel automated polymerase chain reaction-based tool for the diagnosis of tuberculosis. Int J Tuberc Lung Dis 15: 988-989.

27. Malbruny B, Le Marrec G, Courageux K, Leclercq R, Cattoir V (2011) Rapid and efficient detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory samples. Int J Tuberc Lung Dis 15: 553-555.

28. Marlowe EM, Novak-Weekley SM, Cumpio J, Sharp SE, Momeny MA, Babst A, Carlson JS, Kawamura M, Pandori M (2011) Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. J Clin Microbiol 49: 1621-1623.

29. Hasan Z, Arif F, Shakoor S, Mehnaz A, Akber A, Kanji A, Ashraf M, Hasan R (2016) Effective testing for pulmonary tuberculosis using Xpert MTB/RIF assay for stool specimens in immunocompetent Pakistani children. Int J Mycobacteriol 5 Suppl 1: S8-s9.

30. Saeed M, Iram S, Hussain S, Ahmed A, Akbar M, Aslam M (2017) GeneXpert: A new tool for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*. J Pak Med Assoc 67: 270-274.

Corresponding author
Dr. Bilal Aslam, PhD
Department of Microbiology
Government College University, Faisalabad, New Campus,
Jhang Road, Faisalabad-38000, Pakistan
Phone: +92-300-7471091
Fax: +92-41-9203023
Email: drbilalaslam@gcuf.edu.pk

Conflict of interests: No conflict of interests is declared.