Prevalence of Mycobacterium Tuberculosis Strains in both Respiratory and Non- Respiratory Clinical Specimens and their Resistance to Rifampicin at a Tertiary Care Teaching Hospital in Pakistan

Faheem Jan,[1] Muhammad Yasin,[2] Mazhar Ali Khan,[3] Arif Jan,[4] Abdul Samad,[5] Amin Jan,[6] Muhammad Ilyas,[7] Anwar Khalid[8]

[1]Programmatic Management of Drug Resistant TB unit Ayub Teaching Hospital Abbottabad - Pakistan.

[2]Department of Pulmonology, Ayub Medical College Abbottabad - Pakistan.

[3]Programmatic Management of Drug Resistant TB unit Lady Reading Hospital Peshawar - Pakistan.

[4]Faculty of Veternary and Animal Sciences, Gomal University, D. I. Khan – Pakistan

[5]Ecotoxicology Research Program, National Agricultural Research Centre, Islamabad, Pakistan.

[6]Faculty of Pharmacy Gomal University Dera Ismail Khan - Pakistan.

[7]Department of Cardiology, Shaheed Zulfiqar Ali Bhutto University PIMS, Islamabad - Pakistan

[8]Department of Biochemistry, COMSATS University Islam-abad, Abbottabad Campus, Pakistan.

Address for correspondence
Anwar Khalid
Al-Aleem Medical College
COMSATS University Islamabad, Abbottabad Campus, Khyber Pakhtunkhwa Pakistan.
E-mail:
anwar_khalid147@yahoo.com

Date Received: May 12, 2020
Date Revised: July 15, 2020
Date Accepted: August 29, 2020

Author Contributions

FJ MY MAK conceived idea, FJ AK MY AN drafted the study, FJ AJ collected data, MAK AK MI AJ did statistical analysis and interpretation of data, FJ MY AJ AS critical review manuscript, All approved final version to be published.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Abstract

Background: Tuberculosis (TB) is one of the diseases causing high rate of morbidity and mortality worldwide. Amongst high TB burden countries Pakistan ranks fifth, with an estimated new TB cases of 510,000 emerging each year. Worldwide resistance to anti-tubercular drugs is a serious public health issue.

Objective: This study was aimed to determine Mycobacterium tuberculosis (MTB) prevalence both in respiratory and non-respiratory clinical samples, their resistance to the first line Anti-tubercular drug especially to Rifampicin, hence to determine Multidrug resistant Tuberculosis prevalence in the region.

Method: This study was carried out to elucidate Mycobacterium tuberculosis (MTB) prevalence and their Rifampicin resistance among patients of Ayub teaching hospital and nearby districts in Hazara division of Pakistan from July 2017 to September 2019. Total 276 clinical samples were collected (both respiratory and non-respiratory) and screened by Zeihl-Neelsen (ZN) staining initially for the presence of Acid-Fast bacilli (AFB). Samples were further subjected to GeneXpert assay for detection of Mycobacterium Tuberculosis and Rifampicin resistance.

Results: Out of 276 samples, 68 (24.64%) were AFB smear positive while MTB was detected in 28 (20.29%) respiratory samples and 9 (6.52%) in non-respiratory samples. Rifampicin resistance detected in 2 pulmonary samples (1.45%) and nil (0%) in non-respiratory samples. Conclusion: The possible reason of low prevalence rate of resistance to Rifampicin in current study is due to small sample size and mixing of new and retreated cases. Still the study could play its role to help government and health governing bodies in formulating sufficient plans regarding drug resistant TB control.

Keywords: Tuberculosis (TB); Multidrug Resistant TB (MDR-TB); Rifampicin Resistant (RR); Gene Xpert

This article may be cited as: Jan F, Yasin M, Khan MA, Jan A, Samad A, Jan A, Ilyas M, Khalid A. Prevalence of Mycobacterium Tuberculosis Strains in both Respiratory and Non- Respiratory Clinical Specimens and their Resistance to Rifampicin at a Tertiary Care Teaching Hospital in Pakistan. Pak J Chest Med 2020; 26 (4):194-200

Introduction

Tuberculosis (TB) is one of the diseases causing high rate of morbidity and mortality worldwide and a serious health issue. Among high TB burden countries Pakistan ranks fifth, with 510,000 estimated new cases of TB emerging each year.[1] The Global progress of TB control is badly affected by Anti-TB drug resistance, hence it is a serious health problem. Number of reasons are associated with drug resistance including irrational and improper use of Anti-TB drug during chemotherapy. Basically, resistance to anti TB drugs indicates failure of TB control program in that specific locality.[2,3] Different approaches are used for management of patients who are infected with Mycobacterium tuberculosis complex (MTBC) especially with drug resistant TB. For appropriate management early detection, alternate anti tubercular treatment regimens and isolation of patients is necessary.[4] Moreover, to prevent spread of resistant microbes in community, early detection of such cases is given prime importance.

Anti-tubercular medicines are being used for last several decades and resistance is noted to anti-tubercular medicines throughout the world to one or more drugs.[5] In-vitro resistance to Rifampicin (one of the 1st line anti-tubercular drug) showed by bacilli is known as Rifampicin-resistant tuberculosis (RR-TB), requiring second line anti tubercular medications, longer treatment duration, decreased compliance of the patients and occurrence of more adverse effects as compared with Rifampicin sensitive TB. Resistance development to Rifampicin and isoniazid (the 1stline bactericidal anti-tubercular drug) together is known as Multidrug-resistant tuberculosis (MDR-TB).[5]

In 2014, 5% of universally reported cases of tuberculosis were having Multidrug resistant tuberculosis (MDR-TB). Surveillance data reports showed estimation of MDR-TB in 480,000 people out of which 190,000 died. Extensively drug resistant TB (XDR-TB) was reported in 105 countries. On average MDR-TB having XDR-TB was estimated in 9.7% of people.[6]

For MDR-TB detection resistance to Rifampicin is considered as an alternate method, as it was observed in larger population that strains showing resistance to Rifampicin showed resistance to Isoniazid (INH) as well. Although INH and Rifampicin resistance could be detected by genetic testing methods such as Line Probe Assay (LPA) but Drug Sensitivity Testing (DST) and culture identification are gold standard methods and wide range of health professional use these methods in population with higher burden of drug resistant TB.[7]

Methodology

Study Design

This study was carried out to elucidate Mycobacterium tuberculosis (MTB) prevalence and their Rifampicin resistance among patients of Ayub teaching hospital and nearby districts in Hazara division of Pakistan from July 2017 to September 2019. Subsequently molecular detection of MTB species and Rifampicin resistance was studied using GeneXpert. Petroff's approach was used for the decontamination and concentration of all the samples.[5] Primary screening was carried out through Zeihl-Neelsen (ZN) stain. Irrespective of ZN result, all samples were subjected for Molecular detection of Mycobacterium species and subsequently for Rifampicin resistance through GeneXpert.

Patients Selection Strategy

Those patients were selected for this study having Tubercular infection with clinical or radiological suspicion. Our study comprised of total 276 samples (138 for each pulmonary and extra pulmonary). Pulmonary samples consisted of freshly collected non contaminated sputum produced by deep expectoration, broncho-alveolar lavage and Pleural fluid while extra pulmonary samples consisted of Cerebro spinal fluids, Ascitic fluids and Gastric fluids for inclusion in the study. Samples included were from all races of society and irrespective of patients treatment status i.e. newly suspected and post treatment cases. We excluded Non tuberculous Mycobacteria/Mycobacterium other than Tuberculosis (NTM/ MOTT) and all contaminated samples as per Bartlett's grading system determination. Following the standard guidelines for diagnosis of TB those sample were also excluded from the study which contained whole blood as well as swab samples.

Ethical Approval

This study was approved by the institutional review board (IRB), of Ayub Medical Institute, Abbottabad, Pakistan.

Identification of Rifampicin susceptibility and Mycobacterium tuberculosis complex (MTBC):

For Mycobacterium tuberculosis complex (MTBC) detection and resistance to Rifampicin was determined by using a Hemi nested real time Polymerase chain reaction (PCR). The instrument which performs sample processing and subsequent analysis in one step is used for identification of MTBC and for detection of resistance to Rifampicin is Gene Xpert MTB/ RIF assay a novel integrated diagnostic instrument of Hemi nested real time PCR.[8,9] Sequence of DNA specific for Mycobacterium Tuberculosis and resistance to Rifampicin was identified by Gene Xpert MTB/ RIF through PCR.[8,10]

This assay is based on principles of Cepheid GeneXpert system, a platform for effortless and rapid nucleic acid amplification tests (NAAT). Mycobacterium tuberculosis bacilli present in the sputum specimens are purified and concentrated by the Xpert MTB/RIF and subsequently isolates genetic material from the trapped bacteria through sonication and finally amplifies the DNA using PCR.

PCR amplifies 81 base-pairs segment of M. tuberculosis RNA polymerase beta (rpoB) gene detect Rifampicin resistance by probing of this region for mutations. This procedure recognizes all the clinically significant induced Rifampicin resistant mutations in RNA polymerase beta (rpoB) gene in Mycobacterium tuberculosis genomic material in real time using luminous probes known as molecular beacons. Specificity and sensitivity of Gene Xpert is 98.3% and 97.2% respectively (according manufacturer). Due to its quick detection, easy sample processing and almost negligible chance of contamination, Gene Xpert acceptability is high.[8]

Statistical Analysis

The statistical analysis was carried out by simple calculation of arithmetic mean, percentages and one-way ANOVA using SPSS v-20 ®.

Results

Total 276 samples were considered for MTB detection. In our study we checked HIV status of all tuberculosis positive cases by immunochromatographic assay (ICT) and found all of them to be negative for HIV. Initially sputum samples were analyzed for pulmonary MTB detection among 138 samples in which 28 were MTB positive based on Gene Xpert results, while 138 samples of extra pulmonary TB were screened in which 9 were MTB positive. Total 68 samples were AFB positive on microscopy among those only 37 samples (both pulmonary and extra pulmonary) were MTB/ Rif positive on Gene Xpert while rest of 31 samples showed characteristics of MTB but Gene Xpert cleared the doubt that these 31 samples may be infected with other Mycobacterium species than Mycobacterium TB as shown in Figure 2. The rpoB gene (which confers rifampicin resistance) molecular detection results through Gene Xpert are summarized in Table 1. These results show that 20.29% (28) out of total pulmonary samples were Gene Xpert positive for Mycobacterium Tuberculosis Complex (MTBC) and 1.45%[2] samples were Rifampicin while the prevalence of Rifampicin resistance within MTB positive samples was 7.14%. Whereas 6.52%[9] out of total 138 extra-pulmonary samples processed on Gene Xpert were identified as positive for Mycobacterium tuberculosiswith no Rifampicin resistance detected in any sample. Prevalence of MTB detected on GeneXpert was 13.41%[37] in which 1.45%[2] samples of Rifampicin resistance were identified which contribute 0.71% prevalence in total population of the study (Table 1).

Gender based distribution of the study revealed that out of 28 MTBC positive pulmonary cases 20 were males and 8 were females. Whereas among 9 MTB positive extra pulmonary cases 3 case were male and 6 were females. The Prevalence of gender within pulmonary samples was 14.49% male, 5.79% females and the rest were NTM detected samples. On the other hand in extra pulmonary samples 2.17% were male and 4.35% were females. Details percent prevalence of pulmonary and extra pulmonary cases within each age group with respect to both genders is given in Table 2. As cumulative number of both genders i.e. 23 males and 9 females samples were MTB positive within total 276 samples. The prevalence of both genders within each age group with respect to type of disease is given in Table 3. The highest prevalence rate was seen in males (47.83%) of age group 21 to 40 followed by females (42.86%) of the same age group in pulmonary samples. Mostly extra pulmonary cases showed zero prevalence rate i.e. both genders of age group upto 20 and above 60

Table 1. Frequency of MTB detection and Rifampicin resistant cases in pulmonary and extra pulmonary samples

Sample Type	Total Samples	MTB Detected in Xpert MTB/Rif	Rif Resistance Detected
Pulmonary	138	28 (20.29)	2 (1.45%)
Extra Pulmonary	138	9 (6.52)	0 (0%)
Total	276	37 (13.41%)	2 (0.71%)

years while in males of 40 to 60 age group. On the other hand in pulmonary samples zero prevalence was seen in female samples of age group upto 20 and above 60 years. Table 3 also reflects that MTB is most prevalent in the age ranged from 20 to 60 years in both genders predominantly pulmonary MTB.

Both age group and type of disease (pulmonary and extra pulmonary TB) wewe statistically non-significant (P= 0.145) i.e. have no significant difference among age group and type of disease was observed in our data (Table 4). On the other hand, age groups with respect to gender perceiving the disease of either type was statistically significant (P= 0.004) and the gender was statistically insignificant in Lue with pulmonary and extra pulmonary TB infection (Table 5). A statistical summary of one-way ANOVA for study variable is given in Table 4 & 5 with details.

As earlier stated, that Rifampicin resistant cases were only 0.71% in total population and 1. 45% in pulmonary samples both of these cases belonged to age group 21 to 40 years, hence the prevalence of rifampicin resistance within this age group was 100% as shown in Table 6.

Discussion

Studies reported around the world and in our country found variation in the prevalence of drug-resistant TB. We found 37 (13.41%) samples positive for Mycobacterium tuberculosis out of 276 (both pulmonary and extra pulmonary). Whereas Rifampicin resistance prevalence was 1.45% and 0% from all pulmonary and extra-pulmonary samples respectively. Rifampicin mono resistance was 4.69 % during 2011-2012 reported by Lahiri et al.[11] while 0 % Rif resistance was reported by Kumar et al., during 2010-2012 from India.[12]

Studies published from different regions of Pakistan revealed high prevalence of MDR-TB, but commonly the study focused on patients like “first time”, “re-treatment with relapse”, “treatment after failure” and “treatment after default”.[13,14] The most probable reasons of low prevalence of drug resistance in our study may be combining of re-treatment as well as new cases and smaller sample size.

Low prevalence of Rifampicin mono resistance from various parts of the country was reported in many Pakistani studies. Another reason of low rate of Rifampicin resistance in our study could be due to co-

Table 2. Frequency of pulmonary and extra pulmonary patients among different age groups.

Age distribution (in years)	Pulmonary		Extra Pulmonary	
	Male	Female	Male	Female
Upto 20	2 (7.14%)	0	0	0
21-40	11 (39.29%)	6 (21.43%)	3 (33.33%)	4 (44.44%)
41-60	6 (21.43%)	2 (7.14%)	0	2 (22.22%)
Above 60	1 (3.57%)	0	0	0
Total	20 (14.49%)	8 (5.79%)	3 (2.17%)	6 (4.35%)

Table 3. Gender wise distribution among age groups

Gender Type	Upto 20		21-40		41-60		Above 60	
	P	E	P	E	P	E	P	E
Male	8.70%	0.00%	47.83%	13.04%	26.09%	0.00%	4.35%	0.00%
Female	0.00%	0.00%	42.86%	28.57%	14.29%	14.29%	0.00%	0.00%

Table 4. Significance of age group with respect to MTB Types.

Age Groups (years)	Count	Sum	Average	Variance	P-value
Upto 20	2	2	1	2	0.145
21–40	2	24	12	50	
41–60	2	10	5	18	
Above 60	2	1	0.5	0.5	
Pulmonary	4	28	7	54	0.283
Extra Pulmonary	4	9	2.25	10.92	

Table 5: Significance of age groups with respect to gender

Age Groups (years)	Count	Sum	Average	Variance	P-value
Upto 20	2	2	1	2	0.004
21 – 40	2	24	12	8	
41 – 60	2	10	5	2	
Above 60	2	1	0.5	0.5	
Male	4	23	5.75	34.92	0.574
Female	4	14	3.5	22.33	

Table 6: Age and Sex distribution of MTB positive Rifampicin resistant cases.

Age Groups (years)	Pulmonary		Extra Pulmonary	
	Male (n=2)	Female (n = 0)	Male (n=2)	Female (n = 0)
Upto 20	0	0	0	0
21 – 40	2 (100%)	0	0	0
41 – 60	0	0	0	0
Above 60	0	0	0	0

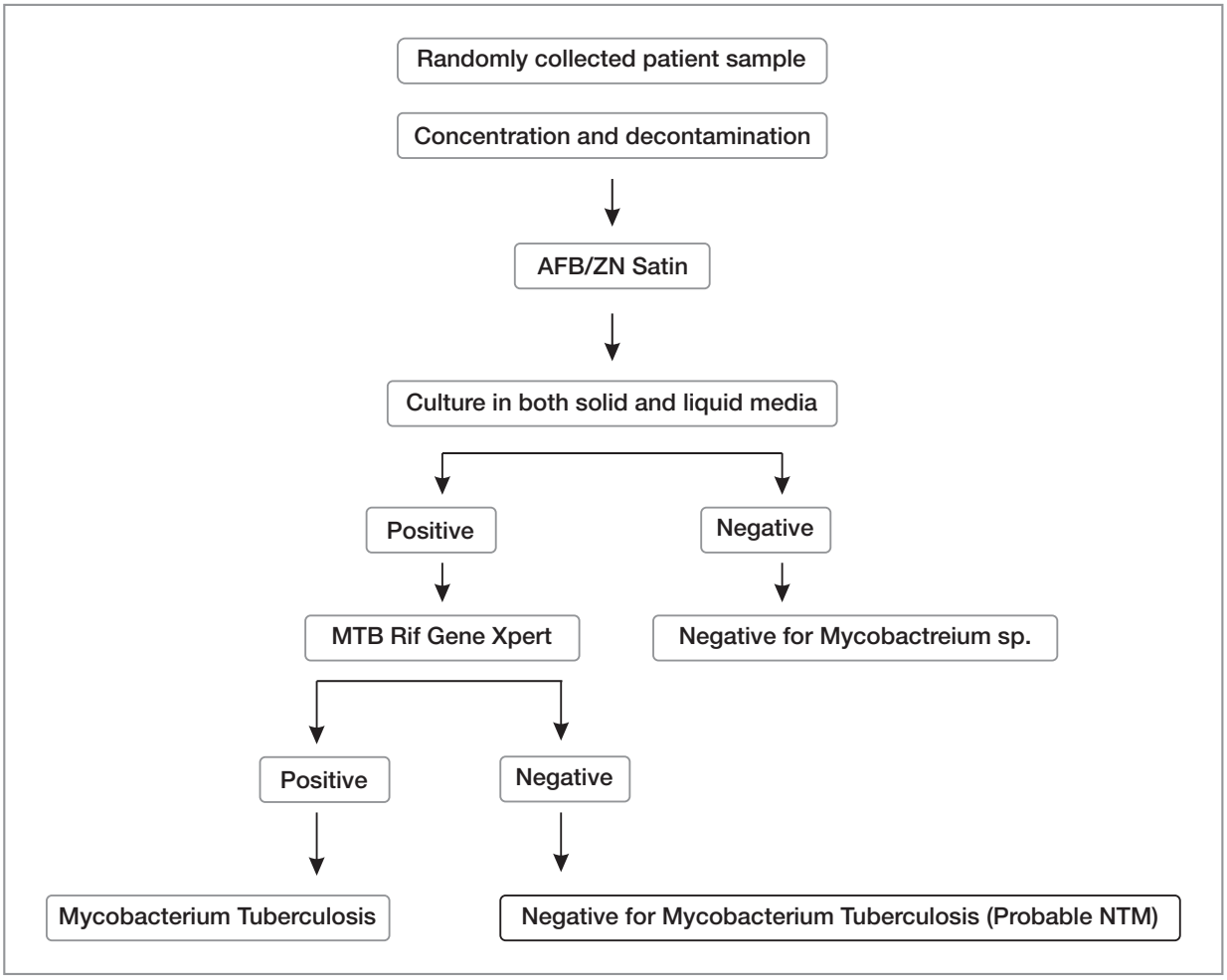

Figure 1: Flow chart of sample processing

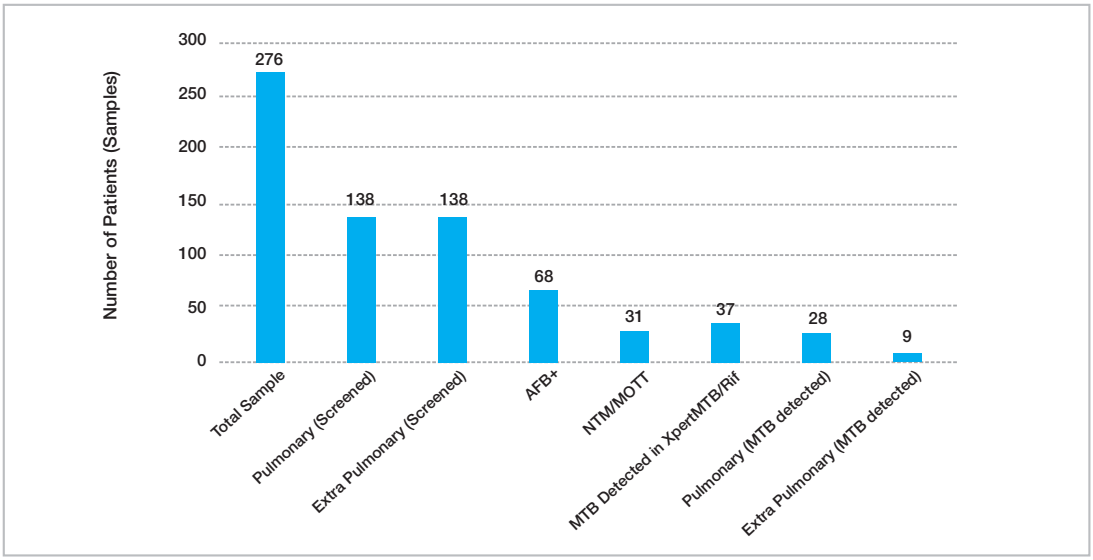

Figure 1: Graphical representation of patients suffering from Pulmonary and extra pulmonary TB

existence of INH resistance and this low rate may act as proxy to the local MDR-TB prevalence.[15,16]

Majority of the male and female patients infected with pulmonary MTB in our study were in the age group of 21-40 years. The age group was also same (21-40 years) in case of extra pulmonary specimen for both males and females (Table 2). Similarly, the detected pulmonary cases of Rifampicin resistance found in males were also in age group 21-40 years. However, due to low sample size we cannot conclude as the statistical values may be insignificant.

We tried to establish an association between drug resistant TB co-infection and HIV. But all TB positive cases were negative for HIV irrespective of their DR-TB status.

The development of resistance to drugs practiced for the treatment of tuberculosis (TB) and particularly multidrug-resistant TB (MDR-TB) has become a substantial public health concern in many countries and also a hurdle in effective TB control.[17] In Pakistan studies conducted in the past over drug resistance surveillance suggest that MDR-TB rate is comparatively higher in Pakistan. However, as management of patients with MDR-TB is inadequate this translates an absolute large number of drug resistant TB cases.

The MDR-TB problem is being addressed by National Tuberculosis Control Program Pakistan (NTP) through taking specific measures including proper management of patients and preventing the transmission and distribution of MDR-TB. Microbial, clinical and programmatic pitfalls are the leading causes of emergence of drug resistant TB. Genetic mutation causes microbial resistance which result in the ineffectiveness of the drugs against the mutant strains of bacilli. These mutant strains become resistant due to poor treatment approaches in TB infected patients. However, poor drug quality, poor adherence and poor treatment strategies all lead to MDR-TB development and it should be stressed that MDR-TB is a man-made phenomenon.[18]

In Pakistan for public health sector Multidrug-resistant tuberculosis (MDR-TB) has become an emerging health issue with lot of challenges. According to the WHO, 3,700 (880- 6,600) cases were MDR retreatment cases among reported pulmonary TB and in 2012, 55 out of 1,602 were laboratory-confirmed MDR retreatment cases.[19] However, among 27 MDR-TB high burden countries globally Pakistan ranks 4th due to number of TB cases reported annually and the size of population.[1]

Throughout the course of this study, we found need for further studies which involve samples of higher number, testing of drug resistant pattern for all first line drugs in future and looking into the prevalence of Non-Tubercular Mycobacterium (NTM).

Conclusion

In Pakistan tuberculosis has been a significant health hazard. Change in the nature of TB with high prevalence of MDR-TB has made it more significant for researcher and practitioners in this field to work on producing regional data. Prevalence study on drug resistant TB from Hazara region could help us at National level to control TB and also help us in this part of country in mapping of drug resistant TB cases. In turn this would also help to plan measures to control TB and tackle cases in such a way to rein or curb in drug resistant TB cases.

References

1. Atif M, Anwar Z, Fatima RK, Malik I, Asghar S, Scahill S. Analysis of tuberculosis treatment outcomes among pulmonary tuberculosis patients in Bahawalpur, Pakistan. BMC research notes. 2018;11(1):370.
2. Hasegawa N, Miura T, Ishii K, Yamaguchi K, Lindner TH, Merritt S, et al. New simple and rapid test for culture confirmation of Mycobacterium tuberculosis complex: a multicenter study. Journal of Clinical Microbiology. 2002;40(3):908-12.
3. Park MY, Kim YJ, Hwang SH, Kim HH, Lee EY, Jeong SH, et al. Evaluation of an immunochromatographic assay kit for rapid identification of Mycobacterium tuberculosis complex in clinical isolates. Journal of clinical microbiology. 2009; 47(2):481-4.
4. Yasmin M, Gomgnimbou MK, Siddiqui RT, Refrégier G, Sola C. Multi-drug resistant Mycobacterium tuberculosis complex genetic diversity and clues on recent transmission in Punjab, Pakistan. Infection, Genetics and Evolution. 2014;27:6-14.
5. Chakraborty S, Chakraborty A, Talukder T, Mukherjee M, Chatterjee T. Prevalence of Mycobacterium tuberculosis strains isolated from both pulmonary and extra pulmonary samples and their resistance to rifampicin: a study from Kolkata and surrounding suburbs. J Tuberculosis Res. 2016;4(2):61-71.
6. Gilpin C, Korobitsyn A, Migliori GB, Raviglione MC, Weyer K. The World Health Organization standards for tuberculosis care and management. Eur Respiratory Soc; 2018.
7. Coovadia YM, Mahomed S, Pillay M, Werner L, Mlisana K. Rifampicin mono-resistance in Mycobacterium tuberculosis in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region. PLoS One. 2013;8 (11):e77712.
8. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. Journal of clinical microbiology. 2010;48(1):229-37.
9. Blakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. Journal of clinical microbiology. 2010;48(7):2495-501.
10. Rie AV, Page-Shipp L, Scott L, Sanne I, Stevens W. Xpert® MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? Expert review of molecular diagnostics. 2010;10(7):937-46.
11. Lahiri S, Mukherjee A, Hazra S, Jana P, Roy S, Saha BK. First-line anti-tubercular drug resistance of mycobacterial strains from re-treatment cases that were smear-positive at 4th month onwards under the Revised National Tuberculosis Control Program. Lung India: official organ of Indian Chest Society. 2015;32(2):127.
12. Kumar M, Rao V, Setty C. Study of prevalence of multi-drug resistant tuberculosis in a tertiary care hospital. Scholars J Applied Med Sci. 2014; 2(1):269-73.
13. Gupta S, Bandyopadhyay D, Sadhukhan S, Banerjees S. A sociodemographic study of multidrug resistant tuberculosis cases from DOTS clinics of Kolkata. Journal of the Indian Medical Association. 2012;110(10):723-5.
14. Almeida D, Rodrigues C, Udwadia ZF, Lalvani A, Gothi G, Mehta P, et al. Incidence of multidrug-resistant tuberculosis in urban and rural India and implications for prevention. Clinical infectious diseases. 2003;36(12):e152-e4.
15. Ramachandran R, Nalini S, Chandrasekar V, Dave P, Sanghvi A, Wares F, et al. Surveillance of drug-resistant tuberculosis in the state of Gujarat, India. The International Journal of Tuberculosis and Lung Disease. 2009;13(9):1154-60.
16. Paramasivan C, Rehman F, Wares F, Sundar Mohan N, Sundar S, Devi S, et al. First-and second-line drug resistance patterns among previously treated tuberculosis patients in India. The International Journal of Tuberculosis and Lung Disease. 2010;14(2):243-6.
17. Chauhan L, Arora V. Management of pediatric tuberculosis under the revised national tuberculosis control programme. Indian journal of pediatrics. 2004;71(4):341-3.
18. Sharma S, Kumar S, Saha P, George N, Arora S, Gupta D, et al. Prevalence of multidrug-resistant tuberculosis among category II pulmonary tuberculosis patients. Indian Journal of Medical Research. 2011;133(3):312.
19. Kanwal S, Akhtar AM, Ahmed A. Factors associated with mortality to drug-resistant tuberculosis and their programmatic management in treatment centres of Punjab, Pakistan. Pakistan J Pak Med Assoc. 2017;67(6):858-62.