Original Research Article

A Review of Rifampicin-Resistant *Mycobacterium tuberculosis* Between 2015 – 2017 in Port Harcourt, Nigeria.

ABSTRACT

Background: The diagnosis and treatment of drug resistant tuberculosis (TB) has presented a unique challenge to the control of TB in Nigeria.

Aim: This study sought to establish the prevalence of rifampicin-resistant TB in our state with a view to advocating for more commitment to efforts toward the treatment and control of drug-resistant TB, including establishment of more treatment centres in the geopolitical zone.

Method: A retrospective review of *Mycobacterium tuberculosis* and Rifampicin resistance detected by GeneXpert™ analysis between 2015 and 2017 in Braithwaite Memorial Specialist Hospital was carried out.

Results: A total of 6733 samples were received and analyzed in the period under review, 1252 (19%) were positive for *Mycobacterium tuberculosis* and 5841 (81%) were negative. The prevalence of *Mycobacterium tuberculosis* was 24.56% in 2015, 20.11% was reported in 2016 and 16.86% was reported in 2017. There was a significant decline in the prevalence of MTB from 2015 to 2017 ($\chi^2 = 33.59$, $p = 0.0001$). In 2015, RIF-resistance was 5.42%, in 2016, it was 5.86% and 6.22% in 2017. There was no significant trend in the Rifampicin-resistance in the period under review ($\chi^2 = 0.21$; $p = 0.6418$).

Conclusion: The study showed that despite a decrease in the prevalence of tuberculosis infection, there was an increase in Rifampicin resistance from 5.42% to 6.22% between 2015 and 2017. There is an urgent need to improve the management of TB in the Port Harcourt metropolis to improve treatment outcomes and prevent the proliferation of drug resistant strains.

Keywords: GeneXpert, Rifampicin-resistance, Trend, Tuberculosis,

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INTRODUCTION

Mycobacterium tuberculosis still remains one of the major public health concern and the cause of several deaths [1,2]. Nigeria ranks the third among the 22 high TB burden countries after India and Indonesia. According to the World Health Organization WHO approximately 480,000 new cases of multidrug resistant TB (MDR TB) occurred in 2014 globally [3]. Tuberculosis (TB) is a major public health challenge with a high mortality rate especially in low and middle-income countries. It is the most common opportunistic infection observed in HIV infected persons with an increased likelihood of mortality [4]. WHO reports that 26% of persons with TB infection in Nigeria are infected with HIV, while the country has the tenth largest TB burden in the world [5-7]. Drug resistant TB is a growing global health problem, posing a challenge to the control of TB, while also prolonging treatment time, limiting treatment options and increases cost of treatment [2,6]. In some settings, drug resistance has been linked with treatment failure and death in 10-30% of TB cases. The estimates based on modeling predict MDR-TB prevalence in

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Nigeria to range from 1.8% (0.0 to 4.3%) for new cases up to 7.7% (0.0 to 18.0%) for previously treated patients (Boehme e*t al*., 2010). Despite antiretroviral therapy (ART), tuberculosis (TB) remains a major cause of morbidity and mortality among persons with human immunodeficiency virus (HIV) infection in Sub-Saharan Africa [2]. Prompt and accurate diagnosis of TB and timely initiation of appropriate treatment decreases TB transmission and mortality [8-10]. To aid prompt TB diagnosis, the World Health Organization (WHO) in 2010 endorsed the Cepheid Xpert® MTB/RIF (Xpert) as a first line tool for diagnosis of HIV-associated TB [6,11]. Xpert is a nucleic acid amplification test that simultaneously detects MTB and rifampicin resistance, and has demonstrated high sensitivity (79.7–100%) as well as shorter diagnostic turnaround time (<2 h) when compared to TB culture [10-12]. As at 2015, 60% of countries recommended Xpert as the initial TB test for persons with possible drug-resistant (DR) TB, and 69% recommended it as the initial diagnostic test in cases of presumptive HIV associated TB [13-15]. In resource poor settings such as Nigeria, drug resistant testing is almost always based on tests from GeneXpert analysis which only detects Rifampicin resistance. The early detection of drug resistance will definitely aid the managing physician for effective treatment of the patient. An assessment of the trend in *Mycobacterium tuberculosis* prevalence and Rifampicin-resistance was assessed in Rivers State, Nigeria.

METHODS

Study Design

A retrospective analysis of the prevalence of TB and rifampicin resistance at the Braithwaite Memorial Specialist Hospital between 2015 and 2017 was carried out.

Study Area

The study was carried out in Port Harcourt, the capital city of Rivers state, Nigeria. The state is an oil rich state. The study was conducted in GeneXpert TB Laboratory at the Braithwaite Memorial Specialist Hospital (BMSH) in Rivers State.

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Study population and Sample

The study population consisted of 6733 presenting with symptoms suggestive of tuberculosis and sent for laboratory diagnosis at the Pathology Department of the Hospital between 2015 and 2017.

Specimen Analysis

Samples were obtained from each informed patient and tested with GeneXpert instrument and was reported as: (1) MTB detected rifampicin resistance detected, (2) MTB detected rifampicin resistance not detected, (3) MTB not detected, or (4) MTB detected Indeterminate. Patients with indeterminate results had the test repeated using fresh samples, and results of the repeat Xpert test were documented as the final Xpert result. Based on the WHO case definition, a patient was classified as having Pulmonary TB if sputum sample was positive by SM or Xpert (bacteriologically confirmed case) or if SM and Xpert were negative but the treating physician made a diagnosis of Pulmonary TB and initiated full TB treatment (clinical diagnosis) [15-17].

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Data Collection and Analysis

Data from laboratory records between 2015 and 2017 was collected and imputed into the Microsoft Excel sheet and analysis. Frequency count and percentage was used to analyze the number of *Mycobacterium tuberculosis* detected by GeneXpert™ . Chi-square for trend was used to analyze the prevalence of *Mycobacterium tuberculosis* and Rifampicin-resistance in the different years of the period under review. All analysis was done with the Epi Info software at a 95% confidence interval and a p-value of less than 0.05 was considered significant.

RESULTS

Figure 1 shows that of the 6733 samples received and analyzed in the period under review, 1252 (19%) were positive for *Mycobacterium tuberculosis* and 5841 (81%) were negative. Figure 3 shows the prevalence of *Mycobacterium tuberculosis* in the period under review, a 24.56% was observed in 2015, 20.11% was reported in 2016 and 16.86% was reported in 2017. There was a significant decline in the prevalence of MTB from 2015 to 2017 ($\chi^2 = 33.59$, $p = 0.0001$). Table 1 shows the trend of Rifampicin resistance in the period under review. In 2015, RIF-resistance was 5.42%, in 2016, it was 5.86% and 6.22% in 2017.

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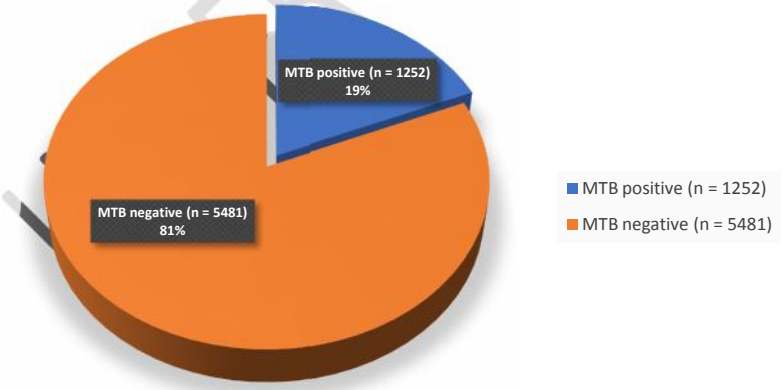

Figure 1: Distribution of MTB Positive samples

Figure 2 shows the distribution of the samples received for *MTB* analysis in the period under review.

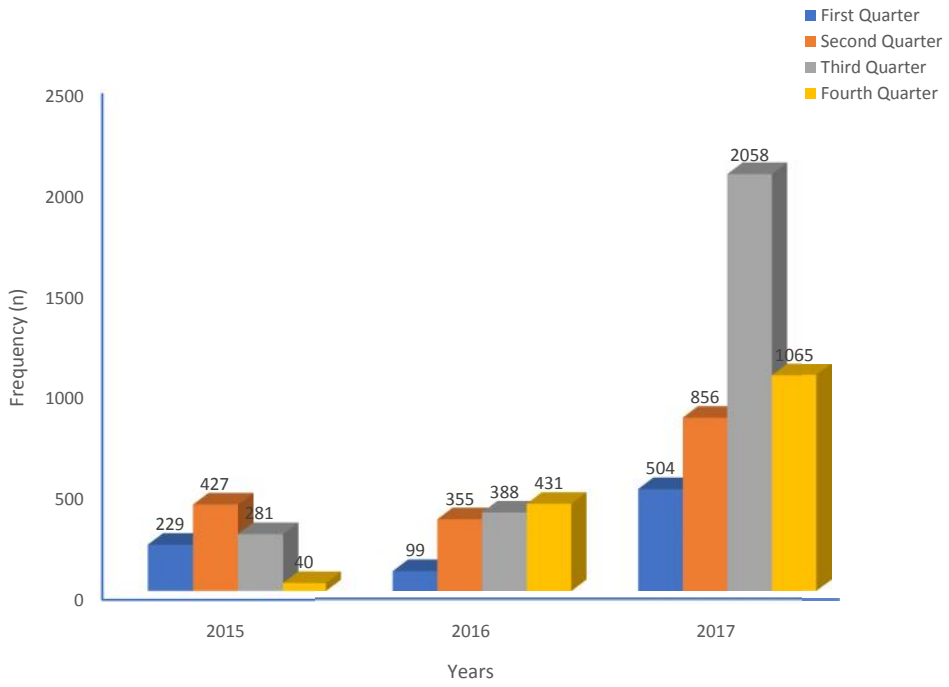

Figure 2: Distribution of Samples Analyzed in the Period Under Review

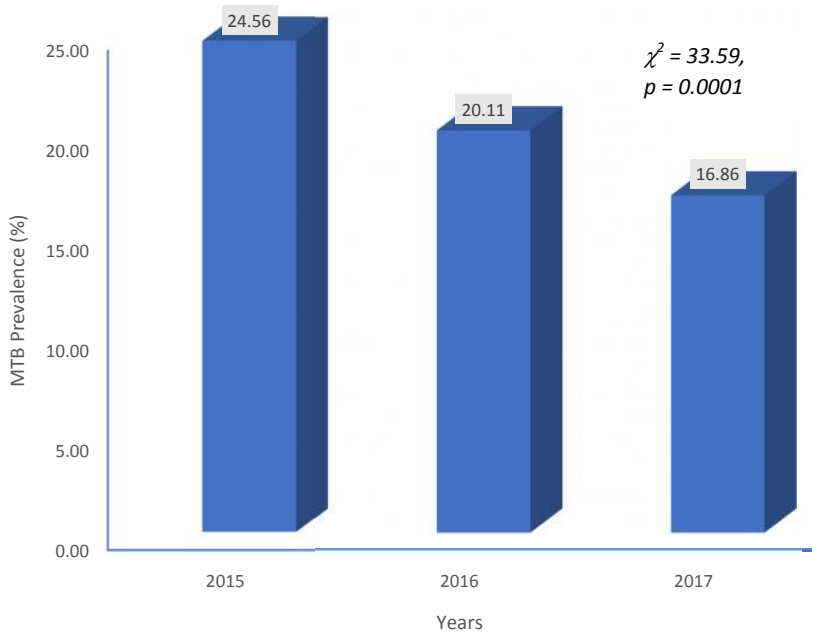

Figure 3: Prevalence of MTB in the Period under review

Table 1: Distribution of Rifampicin Resistance 2015 - 2017

Year	MTB positive (n)	RIF Resistant (n)	% RIF Resistance	χ^2 for trend (p-value)
2015	240	13	5.42	
2016	256	15	5.86	*0.21 (0.6418)***
2017	756	47	6.22	

A bit more detail sociodemographic data on the patients would help

DISCUSSION

Drug resistant TB develops due to inadequate treatment of active pulmonary TB. Inadequate treatment could result due to poor drug selection, lack of adherence to duration of treatment, failure of the appropriate treatment of TB patients. Programmatically this could be from improper prescription of anti-TB treatment regimens, inadequate drug supply, poor quality of drugs, high default and treatment failure rates [18-20].

The average prevalence of TB in the period under review was 20.5%. This is higher than the national average of between 11 – 15% as reported in previous studies (Table 1 ~~shows the trend of Rifampicin resistance in the period under review.~~ In 2015, RIF-resistance was 5.42%, in 2016, it was 5.86% and 6.22% in 2017 [21-26]. This high prevalence may be attributed to the metropolitan nature of Port Harcourt city and the fact that people from neighboring states seek medical attention in the city due to the presence of secondary health care institutions with diagnostic facilities not found in primary health care institutions in the rural and semi-urban areas, a general phenomenon in low and middle-income countries [22, 27]. There was a significant decline in TB prevalence from 24.56% in 2015, to 16.86% in 2017 ($\chi 2 = 33.59$, $p = 0.0001$). This decline corresponds with the reported improvement in TB detection as a result of the increased surveillance mechanisms due to foreign aid and intervention of the TB control program in the country [28-31]. Rifampicin resistance was found to increase from 5.42%, in 2015 to 6.22% in 2017. Prevalence of drug resistant TB among the study subjects which is lower than the previously reported 7.6% in treatment naïve subjects by Dinic *et al*., [17] but considerably lower than the 62.5% reported in South West Nigeria. This prevalence could also be an indication of the prevalence of MDR-TB among the TB infected subjects, which is consistent with the 6% reported by Nwofor *et al*. [31]. but slightly higher than the 5.5% reported by Dinic *et al*. [17]. This is a common occurrence in HIV-prevalent settings where Isoniazid (INH) prophylaxis is used as a preventive measures exacerbating the challenge of multidrug resistance [31, 32]. The overall prevalence of rifampicin resistant mycobacterium tuberculosis in Nasarawa State, Nigerian in a study carried out by Audu *et al*. showed 12.1% among the MTB positive patients who constituted 18.8% of the total analyzed data [4]. The study showed high prevalence of rifampicin resistance (62.8%) among patients from other facilities and 37.2% were from the host facility. The resistance rate was higher in Nasarawa compared to Port Harcourt. The reduced prevalence in the host facility in Nasarawa may be due to the fact that the host facility serves as a referral centre, and suspected drug resistant cases were referred to the Gene Xpert laboratory. This also confirms a report from India that showed that patients referred from facilities outside the facility hosting the laboratory carrying out the diagnosis may have higher prevalence due to selective referrals. Antiretroviral treatment (ART) clinics refer patients more often which also contributes to the lower prevalence in the host facility [34]. A study carried out on 446 sputum specimens sent to TB Referral Hospital Igbogene, Yenagoa Nigeria between January to December 2016 had 102 (22.9%) of the sputum specimens positive for *Mycobacterium tuberculosis*. Out of the 102 positive MTB, 15 (14.7%) showed rifampicin resistance. Their study has established a high prevalence of rifampicin resistance Yenegoa state. Rifampicin resistance in Yenegoa in 2016 was higher than rifampicin resistance for 2015, 2016 and 2017 in Port Harcourt Referral hospital. Other studies from different parts of the world show various prevalence rates. In South Africa, rifampicin resistance ranged between 7.3% and 10%, and from Indonesia, studies showed that 20.5 to 22% culture isolates showed resistance to at least one first-line drug [35-36].

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CONCLUSION

Despite the decrease in the prevalence of Tuberculosis (TB) observed, there was an increase in the occurrence of rifampicin resistance in the period under review. This could be an indication of failed treatment, increase in recurrent infections or the proliferation of drug resistant *Mycobacterium tuberculosis*. There is an urgent need to improve the management of TB in the Port Harcourt metropolis to improve treatment outcomes and prevent the proliferation of drug resistant strains. Keeping to medications, prompt detection and consistent follow up should be adhered to in other to reduce the development and spread of drug resistant *Mycobacterium tuberculosis*. We recommend that ant-TB resistant survey should be carried out on a quarterly bases in all the states in Nigeria.

REFERENCES

1. Alobu, I., Oshi, S.N., Oshi, D. C., Ukwaja, K.N. (2014). Risk factors of treatment default and death among tuberculosis patients in a resource-limited setting. Asian Pac J Trop Med, 7:977–984 pmid:25479627.
2. World Health Organization. Rapid implementation of the Xpert MTB/RIF diagnostic test: technical and operational 'How-to'; practical considerations. Geneva: World Health Organization; 2011.
3. World Health Organization. Global tuberculosis report. 2015. 20th ed. Geneva: World Health Organization; 2015.
4. Audu, E.S., Gambo, M.S., Yakubu, A.A. (2017). Rifampicin resistant mycobacterium tuberculosis in Nasarawa State, Nigeria. Niger J Basic Clin Sci, 14:21-5.
5. Lawn, S.D., Brooks, S.V., Kranzer, K., Nicol, M. P., Whitelaw, A., Vogt, M., Bekker, L. G. (2011). Wood R. Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study. PLoS Med. 8(7): e1001067.
6. Mathew P, Kuo YH, Vazirani B, Eng RH, Weinstein MP. Are three sputum acid-fast bacillus smears necessary for discontinuing tuberculosis isolation? J Clin Microbiol. 2002;40(9):3482–4.
7. Ani, A., Isah, Y., Pwol, R., Lekuk, C., Ashi-Sulaiman, T., Akindgh, M., Akanbi, M., Akande, P., Agbaji, O. (2015). Detection of Mycobacterium tuberculosis by rapid molecular methods augments acid fast bacilli (AFB) smear microscopy in a non-culture tuberculosis laboratory. Afr J Microbiol Res. 9(13):960–4.
8. Lienhardt, C., Rowley, J., Manneh, K., Lahai, G., Needham, D., Milligan, P., McAdam, KPWJ. (2011). Factors affecting time delay to treatment in a tuberculosis control programme in a sub-Saharan African country: the experience of The Gambia. Int J Tuberc Lung Dis. 5(3):233–9.
9. Bassett, I.V., Wang, B., Chetty, S., Giddy, J., Losina, E., Mazibuko, M., Bearnot, B., Allen, J., Walensky, R.P., Freedberg, K.A. (2010). Intensive tuberculosis screening for HIV-infected patients starting antiretroviral therapy in Durban, South Africa. Clin Infect Dis. 51(7):823–9.
10. Boehme, C.C., Nabeta, P., Hillemann, D., Nicol, M.P., Shenai, S., Krapp, F., Allen, J., Tahirli, R., Blakemore, R., Rustomjee, R. et al. (2010). Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med. 363(11):1005–15.

11. Corbett, E.L., Watt, C.J., Walker, N., Maher, D., Williams, B.G., Raviglione, M.C., Dye, C. (2003). The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med. 163(9):1009–21.
12. Coovadia, Y. M., Mahomed, S., Pillay, M., Werner, L., Mlisana, K. (2013). Rifampicin Mono-Resistance in Mycobacterium tuberculosis in Kwazulu-Natal, South Africa: A significant phenomenon in high prevalence Tb/HIV Region. PLos One. 8:e77712
13. Cox, J. A., Lukande, R. L., Lucas, S., Nelson, A. M., Van Marck, E., Colebunders, R. (2010). Autopsy causes of death in HIV-positive individuals in sub-Saharan Africa and correlation with clinical diagnoses. AIDS Rev. 12(4):183–94.
14. Abdurrahman, S.T., Emenyonu, N., Obasanya, O.J., Lawson, L., Dacombe, R., Muhammad, M., Oladimeji, O., Cuevas, L. E. (2014). The hidden costs of installing Xpert machines in a tuberculosis high-burden country: experiences from Nigeria. Pan Afr Med J. 18:277.
15. Abdurrahman, S.T., Mbanaso, O., Lawson, L., Oladimeji, O., Blakiston, M., Obasanya, J., Dacombe, R., Adams, E.R., Emenyonu, N., Sahu, S.(2015). Testing pooled sputum with Xpert MTB/RIF for diagnosis of pulmonary tuberculosis: To increase affordability in low-income countries. J Clin Microbiol. 53(8):2502–8.
16. Zeka, A.N., Tasbakan, S., Cavusoglu, C. (2011). Evaluation of the Gene Xpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extra pulmonary specimens. J Clin Microbiol. 49(12):4138–41
17. Dinic, L., Akande, P., Idigbe, E.O., Ani, A., Onwujekwe, D., Agbaji, O., Akanbi, M., Nwosu, R., Adeniyi, B., Wahab, M, et al. (2012). Genetic determinants of drug-resistant tuberculosis among HIV-infected patients in Nigeria. J Clin Microbiol. 50(9):2905–9.
18. Hanrahan, C.F., Haguma, P., Ochom, E., Kinera, I., Cobelens, F., Cattamanchi, A., Davis, L., Katamba, A., Dowdy, D. (2016). Implementation of xpert MTB/RIF in Uganda: missed opportunities to improve diagnosis of tuberculosis. Open forum Infect Dis. 3(2):068.
19. Helb, D., Jones, M., Story, E., Boehme, C., Wallace, E., Ho, K., Kop, J., Owens, M.R., Rodgers, R., Banada, P., et al. (2010). Rapid detection of mycobacterium tuberculosis and rifampin resistance by use of on-demand. Near-Patient Technol J Clin Microbiol. 48(1):229–37.
20. Otu, A., Umoh, V., Habib, A., Ameh, S., Lawson, L., Ansa, V. (2013). Drug resistance among pulmonary tuberculosis patients in Calabar, Nigeria. Pulm Med. 235190.
21. Rie, A.V., Page-Shipp, L., Scott, L., Sanne, I., Stevens, W. (2010). Xpert® MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? Expert Rev Mol Diagn. 10(7):937–46.
22. Federal Ministry of Health Nigeria: National Guidelines For HIV And AIDs Treatment And Care In Adolescents And Adults. Nigeria; 2010. http://www. who.int/hiv/pub/guidelines/nigeria_art.pdf. Accessed 2 Feb 2017.
23. GeneXpert® Quality Control Features for All Cepheid Xpert® Assays. http://www.swacm.org/workshops/4XpertAssayControl%20Info%20Sheet.pdf. Accessed 8 August 2018
24. Sharma, S.K., Kohli, M., Yadav, R.N., Chaubey, J., Bhasin, D., Sreenivas, V., Sharma, R., Singh, B. K. (2015). Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary *tuberculosis*. PLoS One. 10(10):e0141011
25. Ioannidis, P., Papaventsis, D., Karabela, S., Nikolaou, S., Panagi, M., Raftopoulou, E., et al. (2011). Cepheid Gene Xpert MTB/RIF assay for *Mycobacterium tuberculosis* detection

and rifampin resistance identification in patients with substantial clinical indications of tuberculosis and smear-negative microscopy results. J Clin Microbiol. 49(8):3068–70.
26. John, S., Gidado, M., Dahiru, T., Fanning, A., Codlin, A. J, Creswell, J. (2015). Tuberculosis among nomads in Adamawa, Nigeria: outcomes from two years of active case finding. Int J Tuberc Lung Dis. 19(4):463–8.
27. Kaur, R., Kachroo, K., Sharma, J. K., Vatturi, S. M., Dang, A. (2016). Diagnostic accuracy of Xpert test in tuberculosis detection: A systematic review and meta-analysis. J Global Infect Dis. 8(1):32–40.
28. Mavenyengwa RT, Shaduka E, Maposa I. Evaluation of the Xpert(R) MTB/RIF assay and microscopy for the diagnosis of Mycobacterium tuberculosis in Namibia. Infect Dis Poverty. 2017;6(1):13
29. Nair SA, Raizada N, Sachdeva KS, Denkinger S, Schumacher S, Dewan P, *et al*. Factors associated with Tuberculosis and Rifampicin- Resistant Tuberculosis amongst Symptomatic patients in India: A retrospective analysis. PLos One 2016;11:e0150054.
30. Nek-vorapong R, Sinthuwattanawibool C, Podewils LJ, McCarthy K, Ngamlert K, Promsarin B et al. Validation of the GenoType® MTBDR plus assay for detection of MDR-TB in a public health laboratory in Thailand. BMC Infect Dis. 2010 May 20; 10: 123.
31. Nwofor, A.C., Nyamngee, A. , Nwabuisi, C ., Iwakun, M., Gidado, M. , Mensah, C . et al. (2015). Performance of Genotype MTBDRplus in the Detection of Resistance to Rifampicin and Isoniazid among Clinical Mycobacteria Isolates in Ilorin, Nigeria. Curr HIV Res. 13: 308-314.
32. Steingart, K.R., Sohn, H., Schiller, I., Kloda, L.A., Boehme, C.C., Pai, M., Dendukuri, N. (2013). Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev.1:CD009593.
33. Wiwing, V., Widysanto, A., Lugito, N. P. (2015). *Mycobacterium tuberculosis* Resistance pattern against first-line drugs in patients from urban areas. Int J Mycobacteriol. 4:302-5
34. World Health Organization. The global MDR-TB & XDR-TB response plan 2007-2008.
35. World Health Organization. Stop TB Dept.: treatment of tuberculosis: guidelines. 4th ed. Geneva: World Health Organization; 2010.
36. WHO Tuberculosis Fact sheet No. 104. Reviewed March 2016. www.who.int.org/mediacentre/factsheet/fs104/. [Last accessed on 2016 Apr 04].