THE USE OF GENE XPERT MTB/MTB RIF ASSAY IN THE DIAGNOSIS OF EXTRA-PULMONARY TUBERCULOSIS AT NAUTH, NNEWI

Authors
ANYABOLU Arthur Ebelenna[3], USHIE Simon Nkpeh [1,4], OKONKWO Robert Chuks[1], ENEMUO Emeka Hyacinth[3], UFOAROH Chinyelu Uchenna[3], CHUKUANUKWU Rebecca Chinyelu[2], CHUKWUMA George Okechukwu[2]

Authors' Affiliations

1. TB (DOTS) Laboratory Unit, Department of Medical Microbiology Nnamdi Azikiwe University TeachingHospital, Nnewi.
2. Department of Medical Laboratory Science, College of Health Science, Nnamdi Azikiwe University, Nnewi Campus.

Corresponding Author,
Dr Ushie, Simon Nkpeh
Department of Medical Microbiology and Parasitology,
Faculty of Basic Clinical Sciences,
Nnamdi Azikiwe University, Awka
Nnewi Campus, Anambra State
E-mail: sn.ushie@unizik.edu.ng
+234-8037625333

Abstract

Introduction: About 15–20% of active tuberculosis cases may spread outside the lungs to affect other parts of the body causing what is known as extra pulmonary tuberculosis (EPTB). Cases of Extra Pulmonary Tuberculosis are equally prevalent in all the Tuberculosis high burden zones. Information on Extra pulmonary Tuberculosis is scarce in Nigeria despite being high burden TB country**.** Existing tests for diagnosis of extra pulmonary Tuberculosis are limited in accuracy and turn-around time and often require invasive procedures and special expertise.

Aim: This study aimed to assess the utility of the Gene Xpert MTB/RIF in the diagnosis of extra pulmonary Tuberculosis and document the burden of Extra Pulmonary TB in NAUTH, Nnewi, Nigeria.

Materials and Methods: Extra pulmonary samples (comprising pleural aspirates, ascetic fluids, cerebrospinal fluids, gastric lavage, pus, pleural fluids, scrotal aspirates, hydrocele fluids and biopsy materials) taken from 288 patients accessing the Nnamdi Azikiwe University Teaching Hospital, Nnewi were examined using Ziehl Neelsen smear microscopy and Xpert MTB/RIF assay.

Results: Gene Xpert MTB/RIF assay detected MTB in 18 (6.3%) of 288 EPTB suspects against 8 (2.8%) of 288 detected by Ziehl Nelseen AFB microscopy.
Conclusion: The inclusion of Gene Xpert MTB/RIF assay method in routine diagnostic protocol for EPTB is a welcome development due to its faster turnaround time and higher sensitivity compared to smear microscopy
Key words: ***Extra pulmonary tuberculosis, ZN microscopy, Gene Xpert MTB/RIF, Aspirates***

Introduction

Tuberculosis (TB) is a chronic airborne infection caused by various strains of mycobacterial organisms collectively termed *Mycobacteria tuberculosis* complex. It most commonly (in about 90% of cases) occurs in the lungs as pulmonary tuberculosis (PTB), but in about 15–20% of active cases, the infection may spread hematogenously outside the respiratory system, and affect other organs of the body, causing other kinds of TB generally known as "extra pulmonary tuberculosis" (EPTB). Sometimes both PTB and EPTB could coexist and many organs may be affected simultaneously. The most notable extra-pulmonary infection sites include the pleura (tuberculous pleurisy), central nervous system (tuberculous meningitis), lymphatic system (scrofula of the neck), genitourinary system (urogenital tuberculosis), and the spine bones and joints (Pot's disease), among others [1,2].

Reported prevalence of EPTB varies, among other factors, according to geographical regions, socio-demographic factors and patient sub groups. According to a systematic review by Zumla[3], EPTB occurs in 10-42 % of TB patients depending on the region, ethnic background; age, socio- demographic factors, and immune status of the patients as well as the genotype of Mycobacteria tuberculosis strain; socio-demographic determinants, geographical location, study population, study designs, method of diagnosis are also responsible for the variation in prevalence of EPTB[4]. The presence of co-existing HIV infection has been shown to increase the incidence of EPTB to as high as 62% [5]. The prevalence of EPTB also varies across studies, and it depends on variation of the.

Unlike PTB, EPTB is not infectious [6, 7]. Due to its minimal risk of infection, EPTB receives less attention than PTB. However, the clinical diagnostic challenges and attendant laboratory diagnostic challenges and delayed treatment associated with EPTB gives it a greater potential for morbidity and mortality compared to PTB[4]. EPTB can pose some clinical and laborator

A major challenge in the diagnosis of EPTB is the frequently atypical clinical presentation simulating other inflammatory and neoplastic conditions.y diagnostic challenges. This is most probably why information on EPTB is inadequate. This situation is especially prevalent in many African countries, including Nigeria, despite being one of the 22 countries with highest burden of tuberculosis in the world.

Depending on the organs affected, EPTB can present with a variety of signs and symptoms. Thus, EPTB requires high index of clinical suspicion for its diagnosis[5]. Often, patients are started on anti-tuberculosis treatment empirically based on suggestive symptoms, clinical observations and/or experiences. The laboratory diagnosis of EPTB is also challenging as many forms of EPTB are pauci-bacillary and require invasive diagnostic sampling. Getting adequate specimens can pose a risk of harm to the patient. Clinical samples are obtained from relatively inaccessible sites. This can also decrease the sensitivity of diagnostic tests. Tissue biopsy which is the most effective method of diagnosing EPTB is invasive and sometimes inaccessible. The more easily accessible body fluids, such as pleural, peritoneal, and pericardial fluids, do provide valuable diagnostic clues in EPTB patients, but the number of Mycobacteria in these specimens is often low [8], and usually undetectable with currently available diagnostic methods.

The conventional smear microscopy has a low sensitivity (0%-40%), negative results cannot be definitive[9]. The other available methods also have one or other shortcomings. The reported yields of mycobacterial culture vary from 30% up to 80%, but it usually takes 2-8 weeks to receive the results, such a prolonged turn-around-time is too slow to help treatment decisions. Culture also is cumbersome and requires a highly equipped laboratory and biosafety cabinets level 11/111 which is not commonly available in most of resource limited settings [10].

Tuberculin skin test (TST) and IFN-γ releasing assay (IGRA) may be supportive methods for diagnosing EPTB, but they have limited diagnostic value. The interpretation of TST reactivity can be complicated by cross-reactivity with previous Bacilli Chalmette-Guerin (BCG) vaccination. Factors like HIV infection, poor nutritional status, recent viral or bacterial infections, or vaccination with live virus can reduce response to TST. Like the TST, IGRA cannot distinguish between latent infection and active pulmonary TB or EPTB, and negative results cannot entirely exclude the disease[11]. Diagnosis that is based on histological evidence could also be problematic because loss of host immune function can result in histopathologic findings demonstrating greater suppurative response but less well-formed granulomas[12]. Additionally, the granulomas can be seen also in nontuberculous mycobacterial disease, fungal infections, brucellosis, or syphilis, so cautious interpretation is required[13].

Due to these diagnostic challenges many patients receive the wrong diagnosis. This can lead to unnecessary anti-tuberculosis treatment or poor treatment outcomes or patient not treated at all and subsequently increased morbidity and/or mortality. These problems particularly affect resource-limited settings which usually have high tuberculosis burden[14]. The Gene Xpert MTB/RIF assay method which is a nucleic acid amplification test (NAAT) based on the principle of nested real-time polymerase chain reaction (PCR) has been found useful for the rapid molecular diagnosis of EPTB due to its speed, sensitivity and specificity. A systematic review and meta-analysis had reported that Xpert MTB/RIF has an overall sensitivity of 83.1% and a pooled specificity of 98.7% for the diagnosis of EPTB[15, 16]. Its hands-on operation is easy and requires minimal technical expertise. The results are obtained within a short period of 2 hours. The technique is not prone to cross-contamination and requires minimal biosafety facilities[17, 18]. The World Health Organization[19] formulated new guidelines advocating and recommending the use of Gene Xpert over conventional tests for diagnosis of EPTB[16]. In line with the global recommendations, the National Tuberculosis and Leprosy Control Programme (NTLCP) has recommended the use of Xpert MTB/Rif assay for diagnosis of EPTB in Nigeria[20] and of which the TB (DOTS) center, Nnamdi Azikiwe University Teaching Hospital Teaching Hospital (NAUTH), Nnewi has qued in. It was therefore, the aim of this study to assess the utility of the Gene Xpert MTB/RIF in the diagnosis of EPTB and also document the burden of EPTP in NAUTH, Nnewi.

Materials and Methods

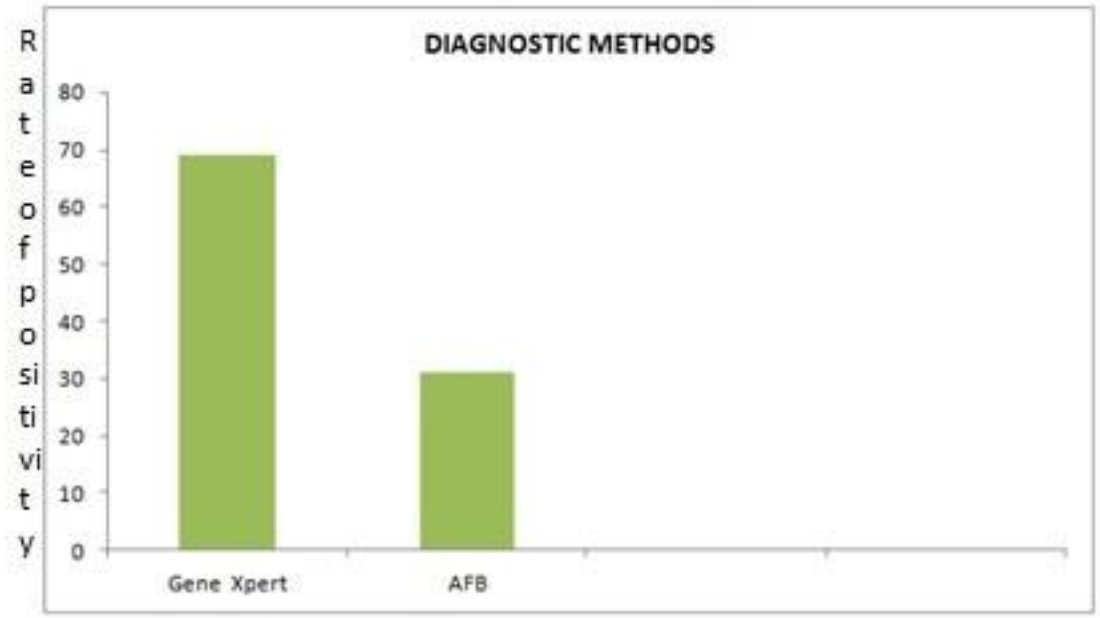
The study population comprises a total of 288 extra pulmonary TB suspects accessing the TB Directly Observed Treatment Short course (DOTS) center of NAUTH, Nnewi between June 2017 and June 2020. Geographically, Nnewi with a population of 391,227 falls within the tropical rain forest region of southeastern Nigeria, and coordinates in latitude $6^0 1' 0'' N$ and longitude $6^0 55' 0'' E$ [21]. The hospital is a tertiary health facility and referral center accessed by patients resident in Nnewi and its environs. Various extra pulmonary samples, comprising 146 pleural aspirates, 50 ascetic fluids, 47 cerebrospinal fluids, 17 gastric lavage, 7 pus, 2 chest fluids, 2 scrotal aspirates, 2 hydrocele fluids and 5biopsy materials, were taken from the patients at different clinics of the hospital based on presenting clinical signs and symptoms and were examined using Xpert MTB/RIF assay and Ziehl Neelsen AFB smear microscopy. Smears measuring 1x2 cm in diameter were made from centrifuged deposits of a portion of the extra pulmonary samples and stained with Ziehl- Neelsen stains. Each stained smear was examined under ×100 oil objective lens and graded according to the International Union Against Tuberculosis and Lung Disease and WHO system: a smear test result was positive if at least one or more acid fast bacilli (≥ 1AFB/100 hpf) was detected[22, 23]. The remaining portion of each sample was diluted 2:1and analyzed by real time PCR process using the Gene Xpert MTB/Rif machine according to the standard operating procedure. The primers and probes in the Gene Xpert MTB/RIF assay use fluorogenic target-specific hybridization to detect the amplified DNA. The results are interpreted from measured fluorescence signals, enabling a semi-quantitative estimate of the mycobacterial load as: MTB not detected (Negative) or MTB detected/RIF resistance not detected (Positive) or MTB detected/RIF resistance detected (MDRTB), or MTB detected Rif resistance indeterminate, accordingly [24].

Results

Gene Xpert MTB/RIF assay detected TB in 18(6.3%) of 288 EPTB suspects against 8(2.8%) of 288 detected by Ziehl Neelsen AFB microscopy as shown in Figure 1. Gene Xpert MTB/RIF detected *Mycobacterium tuberculosis* (MTB) most predominantly in pleural aspirates (15), followed by ascetic fluids(2) and pus aspirates(2) while Ziehl Neelsen smear microscopy detected acid fast bacilli (AFB) in 6 pleural aspirates and 2 pus aspirates respectively as shown in Table 1. The gender distribution showed that males have a higher rate (66.7%) of EPTB infection in this study than females (33.3%).

Table 1 Comparison of Gene Xpert MTB/RIF Assay and AFB microscopy for diagnosis of EPTB.

Sample type	Total no of samples	No positive with Gene Xpert	No positive with AFB microscopy
Pleural aspirate	146	14	6
Gastric lavage	14	0	0
Ascetic fluid	50	2	0
CSF	47	0	0
Pus	7	2	2
Bone marrow aspirate	6	0	0
Oral	3	0	0
Scrotal fluid	2	0	0
Chest fluid	2	0	0
Tissue Biopsy	5	0	0
Hydrocele fluid	2	0	0
Vertebrate fluid	2	0	0
Peritoneal fluid	2	0	0
TOTAL	288	18	8



Discussion

The Gene Xpert MTB/RIF assay is a highly sensitive, specific and fast assay that can now be conveniently used for diagnosis of EPTB. In this study, Gene Xpert MTB/RIF assay detected TB in 9(6.3%) of 144 EPTB suspects. The positivity rate of 6.3% recorded in this study is similar to what were obtained in some few previous studies done in Nigeria as shown here**:** Prevalence rates of 2.9% was previously reported in a study carried out in Anambra and Abia states[25]**,** 5% in Ebonyi state[26]**,** 2.0% in Delta state[27] and 9.5 % in Oshogbo, Western Nigeria[28]. Much higher prevalence rates of 11.4% in Zaria, North Western Nigeria[29], 14.4% in Maiduguri, North Eastern Nigeria[5] and 21.4% in Benin City, South-South Nigeria [30] have been documented. The observed differences could be due to differences in study design, preprocessing methodologies and in input volumes, study populations (adults, children, HIV infected) and using different diagnostic methods of microscopy, gene Xpert or culture. There are also differences bordering on ethnic background, age, underlying disease conditions and genotype of the *Mycobacterium tuberculosis* strains. The methodology and sensitivity of test methods are important issues. For instance, with Gene Xpert MTB/RIF assay which was used for this study, fluid specimens having small sample sizes across a range of various specimen types might be over diluted and could affect the test results. In very pauci-bacillary samples adequate number of bacilli may not be captured and lysed for PCR .Gene Xpert also is reported to be affected by presence of inhibitors to PCR like blood, salts, proteins or cellular debris in fluid specimens. These inhibitors are said to interfere with the amplification enzymes in the PCR process [31].

In this study the positivity rate was higher with Gene Xpert MTB/RIF assay which detected TB in 18(6.3%) of 288 EPTB suspects against 8(2.8%) of 288 detected by Ziehl Nelsen AFB microscopy. Comparable efficacy was observed for EPTB samples in other studies using Gene Xpert MTB/RIF assay and AFB smear microscopy. The overall prevalence of Gene Xpert MTB/RIF assay positive EPTB cases was 8.8% against 2.5% of smear positive cases using fluorescent microscopy in a referral hospital in North Eastern Ethiopia[4]. Also in agreement were other previous studies by Hillemann *et al*[32] and Tortoli *et al* [33]and Shagufta *et al* [34] respectively.

Males have a higher rate (66.7%) of EPTB infection in this study than females (33.3%) A plausible reason for this male predilection may be due to the general trend of males being at greater increased risk for TB acquisition than the females in this environment. The males who are usually the breadwinners are mostly traders and artisans and in this part of the world trading is done in overcrowded environment which could be a predisposing factor for tuberculosis infection[35]. Data on other clinical co-morbidities, socio-demographic and behavioral risk factors for EPTB were not captured in the TB (DOTS) laboratory register and were not considered in this study. Patients' bio data were limited. The low frequencies of the different forms of EPTB in this study may be attributed to the small sample size of this study which in itself could be a result of underutilization of the Gene Xpert MTB/RIF facility by some clinics of this hospital.

Conclusion and Recommendation

In conclusion, Gene Xpert MTB/RIF assay performs comparatively much better than the conventional Ziehl Neelsen smear microscopy as reported variously. This report provides additional local data to support the introduction of the utility of Gene Xpert in the diagnosis of EPTB in Nigeria. The Gene Xpert MTB/RIF assay is a very useful addition to the spectrum of diagnostic methods available for diagnosis of both pulmonary and extra pulmonary tuberculosis. Aside its high sensitive and specificity, it has a highly improved turnaround time (TAT) of about two hours compared to other methods, an advantage that enables the same day diagnosis and the same day treatment.

References

1. *Mazza-Stalder J, Nicod L, Janssens JP. "La tuberculose extrapulmonaire [Extra pulmonary tuberculosis]". Revue des Maladies Respiratoires. 2012; **29** (4): 566–578.*
2. Kumar V, Abbas AK, Fausto N, Mitchel RN. Robbins Basic Pathology (8th edition). Saunders Elsevier.2007; 516-522. ISBN978-1-4160-2973-1.
3. Zumla A., Ranglione M., Hafner R., von Reyn F. Tuberculosis: Current Concept. New England Journal of Medicine. 2014; 368: 745-755.
4. Metaferia Y, Seid A, Fenta GM, Gebretsadik D. Assessment of Extra-pulmonary Tuberculosis Using Gene Xpert MTB/RIF Assay and Fluorescent Microscopy and Its Risk Factors at Dessie Referral Hospital, Northeast Ethiopia. Biomed Res Int. 2018; 7:1-10; 2018:8207098. doi: 10 . 1155 / 2018 / 8207098 . PMID: 30159328; PMCID: PMC6106971.
5. **Goni BW, Bakki B, Saidu IA et al.** Extra pulmonary TB in North Eastern Nigeria: A 10-Year Retrospective Review. Journal of Prevention and Infection Control. 2015; 1:1-10.
6. Sharma SK, Mohan A. Extra-pulmonary tuberculosis. Indian J Med Res. 2004; 120 (4):316-53. PMID: 15520485.
7. *Golden MP, Vikram HR. "Extrapulmonary tuberculosis: an overview". American Family Physician. **2005; 72** (9): 1761–8*
8. **L**awn SD, Zumla AI. Diagnosis of Extra Pulmonary Tuberculosis using Xpert MTB/RIF assay. Expert Review of Anti-infective Therapy. 2012; 10 (6): 631-635.
9. Lee JY. Diagnosis and treatment of Extra Pulmonary Tuberculosis. Journal of Tuberculosis and Respiratory Diseases, 2015; 78(**2**): 47-55.
10. Alvarez-Uria G, Azeona JM, Midde M, Naik PK, Reddy S. Rapid diagnosis of pulmonary and extra pulmonary tuberculosis in HIV-infected patients. Comparison of fluorescent microscopy and the Gene Xpert MTB/RIF assay in a district hospital in India. Tuberculosis Research and Treatment, 2012: 1-4.
11. Fan CZ, Hao XH, Hu ZY, Xio HP. Interferon gamma release assays for the diagnosis of extra pulmonary tuberculosis: A systematic review and meta-analysis. FEMS Immunology and Medical Microbiology, 2012; 65: 456-466.
12. De Noronha AL, Bafica A, Nogueira L, Barral A, Barral-Netto M. Lung granulomas from *Mycobacterium tuberculosis*/HIV-1 co-infected patients display decreased in situ TNF production. Journal of Pathology and Research Practice, 2008; 204:155–161.
13. Zumla A, James DG. Granulomatous infections: etiology and classification. Clinical Infectious Disease Journal, 1996; 23:146–158.
14. *Denkinger CM, Kik SV, Cirillo DM et al. Defining the needs for next generation assays for tuberculosis. Journal of Infectious Diseases, 2015; 211(**2**):29-38.*
15. Kim YW, Kwak N, Seong MW et al. Accuracy of the Xpert(R) MTB/RIF assay for the diagnosis of extra-pulmonary tuberculosis in South Korea. International Journal of Tuberculosis and Lung Diseases, 2015; 19:81–86.
16. Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. European Respiratory Journal, 2014; 44:435–446.
17. World Health Organization (WHO) Nigeria supports introduction of Xpert *MTB*/RIF technology for diagnosis of MDRTB in Nigeria 2012.
18. Evans CA. Gene Xpert- A Game-Changer for Tuberculosis Control? PLoS Med. 2011; 8(7):e1001064.doi:1371/journal.pmed.1001064 .
19. World Health Organization. New technologies for tuberculosis control: a framework for their adoption, introduction and implementation. Geneva, Switzerland 2013.
20. National Tuberculosis, Leprosy and Buruli Ulcer Control Proramme (NTBLCP). Standard Operating Procedure (SOP) for the detection of Mycobacterium tuberculosis complex and Rifampicin resistance from extra pulmonary samples using Gene Xpert MTB/RIF assay in Nigeria. 2015; 1-10.

21. National Population Council (NPC) (2006).

22 International Union Against Tuberculosis and Lung Diseases (IUATLD), technical guide: sputum examination for tuberculosis by direct microscopy in low income countries.5th edition 2000; 10-34.

23. World Health Organization. New technologies for tuberculosis control: A framework for their adoption, introduction and implementation. Geneva, Switzerland, 2007.

24. Small PM, Pai M. Tuberculosis diagnosis-time for a game change. New England Journal of Medicine, 2010; 363:1070-1071.

25. Ogbudebe CL, Chukwu JN, Nwafor CC et al. Reaching the underserved: Active tuberculosis case finding in urban slums in south Eastern Nigeria. International Journal of Mycobacteriology, 2015; 4(**1**): 18-24.

26. Ukwaja KN, Alobu I, Ifebunandu N, Osakwe C, Igwenyi C. From DOTS to the stop TB strategy. DOTS coverage and trend of TB notification in Ebonyi, S.E Nigeria. 1998-2009. Pan Africa Medical Journal, 2011; 9(1): 1-9

27. Eze GU, Adu U, Obiebi IP, Obodo KT. Profile and treatment outcomes of patients with Tuberculosis: A –five year review of patients on DOTS in Delta State, Nigeria. Journal of Community Medicine And Primary Health Care, 2018; 30(**1**):34-48.

28. Olowe OA, Olufunmilola B, Makanjuola OB, Adekanmi AS, Adefioye OJ, Olowe RA. Epidemiological characteristics and clinical outcome of HIV-related Tuberculosis in a population of tuberculosis patients in South-Western Nigeria. European Journal of Microbiology and Immunology, 2017; 7(2):127-132.

29. Oyefabi A, Adetiba E, Lashsk E, Adesigbin O. Tuberculosis and the determinants of treatment outcomes in Zaria, North Western Nigeria: A nine year (2007- 2015) epidemiological review, Journal of Tropical Medicine 2017; 19: 116-122.

30. Affusim CC, Kesieme E, Abah VO. The pattern of presentation and prevalence of tuberculosis in HIV- Sero-positive patients seen at Benin City, Nigeria. International Scholarly Research Notices Pulmonary, 2018:103-108.

31. Suzana S, Ninan MM, Gowri M, Venkatesh K, Rupali P, Michael JS. Xpert MTB/Rif for the diagnosis of extra pulmonary tuberculosis- an experience from a tertiary care centre in South India. International Health, 2015; 21(**3**): 1-16.

32. Hillemann D, Rusch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extra pulmonary tuberculosis by the automated Gene Xpert MTB/RIF system. Journal of Clinical Microbiology, 2011; 49(**4**): 1202-1205.

33. Tortoli E, Russ C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M *et al*. Clinical validation of Xpert MTB/RIF for diagnosis of extra pulmonary tuberculosis. European Respiratory Journal, 2012; 40: 442-447.

34. Shagufta I, Asyia Z, Shahida H, Noshin WY, Maleeha A. Rapid diagnosis of tuberculosis using Xpert MTB/RIF assay-Report frm a developing country. Pakistan Journal of Medical Science. 2015; 31(**1**): 105-110.

35. Agbaji O, Ebonyi AO, Meloni ST, Anejo-Okpoki JA, Akanbi MO, et al. Factors Associated with Pulmonary Tuberculosis-Co-Infection in Treatment-Naïve Adults in Jos, North Central Nigeria. J AIDS Clin Res. 2013;**4**:222.doi:10.4172/21155-6113.1000222kl;