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Review

Review on convectional and advanced diagnostic techniques of human tuberculosis (TB) in Ethiopia

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Tuberculosis is a major health problem throughout the world causing large number of deaths, more than that of any other single infectious disease. Accurate and early diagnosis of tuberculosis is important for its effective management. The continued development of affordable, sensitive, and specific diagnostic tools will be required for use in resource-poor settings, where the incidence of disease is the highest like Ethiopia. This paper was done to give concise review on various convectional and advanced diagnostic techniques of human tuberculosis, focused on diagnostic techniques used in clinical and research setting in Ethiopia.

Key words: Convectional diagnostic, advanced diagnostic, human tuberculosis, Ethiopia.

INTRODUCTION

Tuberculosis (TB) is one of the major public health problems worldwide that affects all age groups. In 2012 alone, there were 8.6 million new cases and 1.3 million deaths globally (World Health Organization [WHO], 2013). It continued to be the leading cause of death globally despite the availability of reliable diagnostic approaches and effective drugs for over decades (Meaze, 2005). It is caused by the genus of *Mycobacterium* which includes many pathogens known to cause serious diseases in mammals (Palomino et al., 2007). TB is mainly caused by the member of *Mycobacterium tuberculosis* complex (MTC) (Singh et al., 2000). Despite the different species tropisms, the MTC is characterized by 99.9% or greater similarity at the nucleotide level and possess identical 16S rRNA sequence (Dye et al., 2005; Smith et al., 2006).

The global distribution of TB cases is skewed heavily toward low-income and emerging economies. Ninety-five percent of all cases and 99% of deaths due to TB occur in developing countries with the greatest burden in sub-Saharan Africa and South East Asia (WHO, 2013). Ethiopia is one of the highest TB endemic countries in the world where the disease is the leading cause of mortality and morbidity. Ethiopia ranks 9th among 22 high TB burden countries and one of the top three in Africa. The disease is affecting large segments of the population in

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the country (Birhanu et al., 2013). In 2012, the estimated annual incidence and prevalence of all forms of TB were 230 and 224 per 100,000 populations, respectively (WHO, 2012). Due to limited diagnostic facilities at hand in developing countries including Ethiopia, much of the diagnosis depends on clinical expertise. However, there is a growing perception for effective diagnosis of TB that no single method is sufficient for detecting all TB patients (Sharma and Gupta, 2011). Thus, the objective of this review paper was to give concise review on various convectional and advanced diagnostic techniques of human TB, focused on diagnostic techniques used in clinical and research setting in Ethiopia.

DIAGNOSTIC TECHNIQUES OF HUMAN TB

Clinical examinations

These are the primary TB diagnostic methods which help for TB case detection in human. The detection strategies include identification of suspects among patients who present on their own initiative at health facilities or in the community. A person is a suspect of pulmonary TB when presenting with persistent cough for two weeks or more. Cough is usually with expectoration, with or without blood stained sputum and can be accompanied by one or more of the symptoms like weight loss, chest pain, shortness of breath, intermittent fever, night sweats, loss of appetite and fatigue and malaise. Every adult patient with respiratory symptoms attending the health facility must be asked about symptoms suggestive of TB, with particular attention to cough persisting for 2 weeks or more. In human immunodeficiency virus (HIV)-positive patients (or in presence of a strong clinical suspicion of HIV-infection), only one positive smear result is necessary to make diagnosis of smear-positive pulmonary TB (FMOH, 2008).

The signs and symptoms of extrapulmonary TB (EPTB) depend mainly on the organ(s) involved. The most common forms and their respective presentations are TBLN is slowly developing and painless enlargement of lymph nodes, followed by matting and eventual drainage of pus; tuberculous pleurisy is painful while breathing in, dull lower chest pain, intermittent cough, breathlessness on exertion; TB of bones and/or joints is localized pain and/or swelling, discharge of pus, muscle weakness, paralysis, stiffness of joints; intestinal TB is loss of appetite and weight, abdominal pain, diarrhoea or constipation, mass in the abdomen, fluid in the abdominal cavity (ascites); and tuberculous meningitis is headache, fever, vomiting, neck stiffness and mental confusion of insidious onset (FMOH, 2008).

Histo-pathological examination

This technique is based on histological examination and samples can be taken from the following: fine needle aspiration of the lymph nodes, affected peripheral lymph nodes, particularly cervical nodes, can be aspirated. Effusions of the serous membranes can be aspirated. However, the liquid aspirated is much less useful for diagnosis than histology and culture of a pleural (membrane) biopsy specimen. Tissue biopsy (serous membranes, skin, endometrium, bronchial, pleural, gastric or liver tissue), can be taken, with or without surgery; surgical intervention can be performed to confirm diagnosis of TB by sampling of a deep or superficial lymph node, a bone fragment or part of an organ. Post mortem, after death from an unknown cause, tissue samples taken at autopsy can be analyzed. Due to the scarcity of facilities for histo-pathological services, this procedure is not routinely practiced all health facilities except in certain referral and specialized hospitals in Ethiopia (FMOH, 2008).

Radiological examination

For the diagnosis of pulmonary TB, X-ray is sensitive but less specific, because abnormalities identified on a chest X-ray suggestive of TB may also be caused by a variety of other conditions. But, there is no radiological finding typical for TB. Many lung diseases can have similar radiological findings mimicking pulmonary tuberculosis (PTB) (Petra, 1994). Moreover, it cannot differentiate active TB from inactive or cured TB. Suggestive X-ray findings are upper lobe infiltrates (bi-lateral or uni-lateral right), cavitations (with a thick wall >2 mm) and patchy, nodular shadows around the cavity. No shadow is typical for TB, and 40% patients diagnosed as having TB by X-Ray alone may not have active TB disease. Some individuals, in fact, who had TB in the past that has been cured (and therefore do not require treatment) may still have a chest X-ray suggestive of active TB. Chest radiography is useful for differential diagnosis of pulmonary disease among patients with negative sputum smears, military and childhood TB, when interpreted in conjunction with presenting signs and symptoms. Readings of chest X- rays should, whenever possible, be made by a radiologist or an experienced physician (Asnake and Feleke, 2000).

Smear microscopic examination

This is a relatively simple, rapid and inexpensive test among the currently available tests utilized for the detection of MTC and other acid fast bacilli (AFB). It has been used as a standard diagnostic method for PTB worldwide, especially in low to middle income countries where about 90% of TB cases are found. It can be used for diagnosis, monitoring and defining cure. Therefore, this is the key diagnostic tool used for case detection

(FMOH, 2008). Every individual suspected of having PTB must have an examination of 3 (spot-early morning-spot) sputum smears, to determine whether or not they have infectious TB. According to the latest recommendation by WHO and the national AFB microscopy laboratory manual, the result of the sputum PTB+ is confirmed when at least 2 out of three smear results are positive for AFB. In HIV-positive patients (or in presence of a strong clinical suspicion of HIV-infection), only one positive smear result is necessary to make diagnosis of smear-positive pulmonary TB (FMOH, 2008).

The smear microscopy has specificity over 95% in a high prevalence setting, but the sensitivity is variable, ranging from 40 to 80%. Sensitivity is even much lower in the case of co-infections with HIV, childhood where sputum yield is very low, and in the case of extra-PTB (FMOH, 2008). High bacterial load per milliliter of sputum (5000 to 10000 ml^{-1}) is needed and it requires good training and constant supervision of the laboratory technicians.

In addition, the smear microscopic method had limitation on its inability to differentiate MTC from NMTC (Non Mycobacterium tuberculosis Complex) species, ability to detect only the actively growing population of the bacilli (about 104 bacilli/ml of sputum) (American Thoracic Society, 1990) while pauci-bacillary cases often turn out negative, ability to detect AFB of other microbial genera such as Norcadia species which may give false positive findings (Kent and Kubica, 1985). To improve the sensitive shortcomings of the smear TB diagnosis, recent techniques had been developed. These include the development of illumination systems based on light-emitting diodes (LEDs), which resulted in LED fluorescence microscopy (LED-FM) usage (Cuevas et al., 2011).

The following techniques have been practiced in routine health facilities in Ethiopia. Proper diagnosis through examination of sputum of patients with symptoms suggestive of TB; promotion of awareness in the community, amongst the medical staff and the community workers regarding respiratory symptoms, notably persistent cough for two weeks or more, and the need to obtain and examine three sputum specimens for the diagnosis of TB; contact screening: examination of household contacts of smear-positive TB patients, irrespective of the duration of cough and intensified TB screening in high-risk groups (FMOH, 2008).

IMMUNOLOGICAL TEST

Tuberculin skin test (TST)

The immunological diagnosis of TB has been historically performed by the Mantoux test or TST, which consists in the intradermic inoculation of the purified protein derivative (PPD) and in measuring 48 to 72 h latera delayed-type hypersensitivity which measure the cell mediated immune response against the75 tubercle bacilli (Lecouvet et al., 2004).

TST is used for diagnosing pediatric TB, in whom a positive test is more likely to reflect recent infection with TB and indicates a higher risk of developing TB disease. TST has been widely used as a screening test in many countries and has contributed substantially to generate valuable epidemiological information on infection trends and on the magnitude of the disease (Harstad et al., 2009; Parenti et al., 1987). Moreover, TST is a valuable tool for the screening of household contacts and suspected cases of TB in BCG vaccinated subjects and in population with high BCG vaccination coverage (Adeyekun et al., 2010).

According to the traditional cut-off points of PPD set by WHO and Centers for Disease Control (CDC), a reaction of ≥10 mm in duration should be considered positive for those persons with an increased probability of recent infection or with other clinical conditions that increase the risk of TB (e.g. recent immigrants from high prevalence countries and injection drug users) (Mohamed et al., 2011). However, in a recent study conducted in Ethiopia a large number of individuals who had skin indurations <10 mm were found positive for latent TB infection by QuantiFERON-TB Gold In-Tube and tuberculin skin test. Most immigrants, including those from Ethiopia have skin in duration of ≥10 mm, but the numbers of immigrants who develop clinical TB are very few. Therefore, considering the effect of environmental *Mycobacterium*, immune suppresser that lower TST response such as HIV/AIDS, diabetes mellitus, cancer diseases, protein energy malnutrition and taking steroid drugs for longer period, and BCG vaccination in most developing countries (Legesse et al., 2011), including Ethiopia, the cut-off point recommended by WHO for screening of TB infection require re-assessment.

Interferon-gamma release assays (IGRAs)

IGRAs are more sensitive and specific immunological assays designed for the immunological diagnosis of *M. tuberculosis* which is a surrogate of TST (Lecouvet et al., 2004). It measures the interferon-γ cytokine released by T-cells obtained from a blood sample following re-stimulation with *M. tuberculosis* antigens. These antigens are specific for MTC and few other mycobacteria such as *Mycobacterium kansasii* and *Mycobacterium szulgai* and are not found in atypical mycobacteria and in the vaccine strain BCG, which lacks the region of difference 1 (RD1), encoding in MTC for these antigens (Diel et al., 2011). IGRAs measure the presence of effector T cells in the blood that are specific for *M. tuberculosis*. Effector T cells can readily respond to an antigenic stimuli by secreting cytokines, and are different from memory T-cells that require more time (>24 h). Effector T cells are present only when the immune system is currently exposed to the

antigenic stimuli which in this case is *M. tuberculosis*. Positive IGRAs gives therefore an indication of an infection, but cannot distinguish similarly to TST, between an active TB from a latent infection. The Quantiferon TB Gold In Tube (QFT-IT) is an IGRAs that measure the amount of interferon-γ secreted following re-stimulation, and many laboratories provide to the clinicians the results in UI/ml (Delogu et al., 2012). Many researchers have attempted to correlate the amount of interferon-γ secreted with TB status, but so far unclear correlation has been found. However, IGRA seems to be a beneficial tool for TB diagnosis, especially for people with a high-risk of developing active TB.

Lymphocyte proliferation assay

This assay compares the reactivity of peripheral blood lymphocytes to tuberculin PPD-B and PPD-A. They can be performed on whole blood or purified lymphocytes from peripheral blood samples. This test endeavors to increase specificity of the assay by removing the response of lymphocytes to “non-specific” or cross-reactive antigens associated with non-pathogenic species of mycobacteria to which the animal may have been exposed. Results are usually analyzed as the value obtained in response to PPD-B minus the value obtained in response to PPD-A. The assay has scientific value, but is not used for routine diagnosis because the test is time-consuming and the logistics and laboratory execution are complicated, meaning it requires long incubation times and the use of radio-active nucleotides. As with the IFN-test, the lymphocyte proliferation assay should be performed shortly after blood is collected. The test is relatively expensive and has not been subjected to inter-laboratory comparisons (Lilenbaum and Fonseca, 2005).

DETECTION OF LIPOARABINOMANNAN

An interesting tool relies on the detection of lipoarabinomannan (LAM), which is a cell wall lipopolysaccharide antigen of MTC. It has demonstrated variable sensitivities in diagnosing TB. A recent study has demonstrated that urinary LAM appears to be related to host immune factors and that it declines steadily after two weeks of anti-tuberculous treatment (Ramachandran and Paramasivan, 2003; Wood et al., 2012). LAM-ELISA may be a suitable option for the diagnosis of HIV-associated TB in urine specimens from patients with low CD_4 cell counts. The sensitivity and specificity of the LAM-ELISA for TB patients versus Ethiopian non-TB patients were 74 and 86.9%, respectively; the positive and negative predictive values were 58.5 and 93.0%. This study suggests that detection of LAM in the urine of TB patients may improve case finding and that diagnostic tests based on this principle may serve as valuable supplemental tools in TB control (Tessema et al., 2001).

Culture method

Mycobacterium culture test provides a definitive diagnosis of TB and more sensitive than smear microscopy as it is able to detect as few as 10 bacilli/ml of digested concentrated clinical specimen (American Thoracic Society, 1990). The culture techniques could be automated or not as the following.

In no automated culture method

Growth detection and identification of MTC may take several weeks. Drug susceptibility testing of a TB isolate requires an additional 1 to 3 weeks. Slow growth of mycobacterial strains (a common characteristic noted in many MDR-TB strains) further lengthens the time to identification and susceptibility testing. Therefore, delay in the return of reports of culture confirmation and susceptibility results will delay the diagnosis of drug-resistant TB and initiation of appropriate treatment.

The egg based Lowenstein-Jensen and stone brinks media are non-automated culture method most commonly used in medical laboratory. In the isolation of mycobacteria by culture, the ideal medium should be able to support rapid and luxuriant growth, and allow the determination of its characteristic features, e.g. colony morphology, growth rate and pigment production (Ang et al., 2001). Most mycobacterial culture media fall into egg-potato-base media and agar-base media (American Thoracic Society, 2000). The most popular egg-based media are the Lowenstein-Jensen buffered egg-potato medium and the American Trudeau Society egg yolk-potato flour medium. Among the agar based media, Middle brook 7H10, Middle brook 7H11, and Dubose oleic-albumin agar are recommended. The advantages of egg-based media are the long shelf life (1 year when refrigerated) and the low cost of preparation. Egg media require heat for solidification, which, along with the presence of albumin, inactivates certain antituberculous drugs. Lowenstein-Jensen medium which contains glycerol is used for the isolation of *M. tuberculosis* but glycerol is inhibitory for most strains of *Mycobacterium bovis* (Grange et al., 2000).

The media can be made more selective by the addition of cycloheximide (400 µg/ml), lincomycin (2 µg/ml) and nalidixic acid (35 µg/ml). Each new batch of culture medium should be inoculated with the stock strains of *Mycobacteria* to ensure that the medium supports satisfactory growth. The inoculated media may have to be incubated at 37°C for up to 8 weeks and preferably for 10 to 12 weeks with or without carbon dioxide for the mycobacteria in the TB group. *M. tuberculosis* and *Mycobacterium avium* prefer the caps on the culture

media to be loose while *M. bovis* grows best in airtight containers (Fentahun and Luke, 2012).

Colonial morphology

The luxuriant growth of *M. tuberculosis* on glycerol containing media, giving the characteristic 'rough, tough and buff' colonies known as eugenic, while the growth of *M. avium* on media containing glycerol is also described as eugenic. *M. bovis* has sparse, thin growth on glycerol containing media that is called dysgenic; however, grow well on pyruvate-containing media without glycerol (Patterson and Grooms, 2000).

AUTOMATED CULTURE METHODS

Automated culture methods are designed to yield results of primary cultures and drug susceptibility tests within the shortest possible period (4 to 21 days). Their ability to combine efficiency and reproducibility overcomes most of the limitations of the conventional methods. Automated machines are commercially available from different manufacturers. These include BACTEC 460 TB System (TB BACTEC), a radiometric procedure which measures $^{14}C_{02}$ released during metabolism of ^{14}C-fatty acid substrate by growing bacteria. The amount of $^{14}C_{02}$ released is expressed as growth index (GI) on a scale of 0 to 999. In the presence of an antimicrobial agent, inhibition of daily GI is considered as susceptibility of test organism to the drug (Sidiqi et al., 1981; Heifets, 1991). The handling and disposal of radioactive medium in this technique is however considered a disadvantage.

The mycobacteria growth indicator tube (MGIT) 960 (Becton Dickinson USA) uses a Middlebrook 7H9 broth in 7 ml plastic tube and a fluorescence quenching-based oxygen sensor embedded at the bottom of the tube. The procedure involves initial, fluorescence quenching by the presence of dissolved oxygen in the broth. Subsequently, growing mycobacterial cells in the inoculated tubes consume the oxygen, producing fluorescence under 365 nm UV illumination. The system continuously monitors the tubes to detect increase in fluorescence and automatically determines and interprets results. When used for drug susceptibility test, a set of drug containing and drug free (growth control) media are inoculated with the test strain. Comparison of records in the test and control tubes are automatically done and reported as susceptible or resistant (Hanna et al., 1999; Tortoli et al., 2000).

Biochemical tests

After a mycobacterial isolates have been classified to a subgroup on the basis of pigment production, colonial morphology and growth rate, specific identification is accomplished using different biochemical tests. Final identification is based on more than one test because individual strains may deviate from the expected results. These tests include nitrate reduction, pyrazinamidase, thiophene 2 carboxylic acid hydrazide and urease tests.

Nitrate reduction test

Mycobacteria differ quantitatively in their abilities to reduce nitrate and the test measures the ability of a strain to produce the enzyme nitrate reductase. It is valuable for the identification of some mycobacteria that possess similar characteristics of colony morphology, growth rate and pigmentation. *M. tuberculosis, M. kansasii, M. szulgai* and most rapid growers are nitrate reductase positive (Virtanen, 1960).

Thiophene 2 carboxylic acid hydrazide (TCH) test

This test is used to distinguish niacin positive *M. bovi*s from MTC and other non chromogenic slowly growing mycobacteria. *M. bovis* is susceptible to low concentrations (1 to 5 g/ml) of TCH where as MTC and other mycobacteria are resistant to the inhibitory action of this compound (Vestal and Kubica, 1967).

Pyrazinamidase test

The enzyme pyrazinamidase hydrolyses pyrazinamide to pyrazinoic acid. This acid is detected by the addition of ferrous ammonium sulfate to the culture medium. The formation of a pink ferrous- pyrazinoic acid complex indicates a positive test. This test is most useful in separating *M. tuberculosis* from *M. bovis* (Wayne, 1974).

MOLECULAR DIAGNOSTIC TECHNIQUES

Polymerase chain reaction (PCR)

With the advance of molecular diagnosis, various PCR methods in diverse clinical specimens have been introduced to identify MTC more easily and quickly. Owing to the limitations of the traditional microbiological methods, puacibacilary nature of the specimen and the extensive differential diagnosis in extra-pulmonary TB, a rapid, sensitive and specific diagnosis is needed in developing countries. It has several advantages over culture, including confirmation of the presence of MTC within one to three days as compared to six weeks with conventional culture techniques (Palomino et al., 2007). Additional advantages of PCR over conventional methods

include its high sensitivity, performance in few hours, and depending on the assay design, ability to differentiate between MTC and mycobacterial species other than TB, and identification of gene mutations associated with drug resistance (FMOH, 2008).

Observing MTC in tissues or smears using ZN staining or fluorescence method allows faster diagnosis. Unfortunately, these methods are insensitive and non-specific. This is the cause for development of a new and sensitive diagnostic technique like PCR. Key mycobacterial targets for PCR amplification are: the insertion sequence (IS) IS6110, 65 KD (kilodalton) heat shock protein, 38 KD protein, and ribosomal RNA. IS6110 is considered to be a good target for amplification as this is found in almost all members in high copy number in most strains of the MTC (Palomino et al., 2007). PCR methods allow direct identification of the MTC and can detect less than ten bacteria in a clinical specimen. PCR's sensitivity ranges from 70 to 90% compared to the results of culture and its specificity varies between 90 and 95%. In smear of positive cases, the sensitivity of PCR is greater than 95%, but in smear of negative cases, it is only 50 to 60%. Therefore, at present, amplification methods should not replace diagnostic convectional culture (Sharma and Gupta, 2011). It has been widely evaluated for the detection of MTC in clinical samples, mainly sputum in human patients and has recently been used for the diagnosis of TB (Ameni et al., 2010).

Spoligotyping

Spoligotyping, also called spaceroligonucleotides typing, is a novel new method for simultaneously detection and typing of MTC bacteria, and has been recently developed. This method is based on PCR amplification of highly polymorphic direct repeat (DR) locus in the MTC genome. The DR region in *M. bovis* BCG contains direct repeat sequences of 36 bp, which is interspersed by the non-repetitive DNA spacers of 35 to 41 bp in length. Other MTC strains contain one or more IS6110 elements in DR-region (Sharma and Gupta, 2011).

The spoligotyping applied to culture is simple, robust and highly reproducible (Soolingen, 2008). The results can be obtained from MTC culture within one day. Thus the clinical usefulness of spoligotyping is determined by its rapidity, both in detecting causative bacteria and in providing epidemiologic information on strain identities. It can also be useful for identification of outbreak and can facilitate contact tracing of TB. PCR based methods are available as diagnostic and confirmatory test for species diagnosis of TB and are expected to detect as low as 1 to 10 organisms (Sharma and Gupta, 2011). Implementation of such a method in a clinic setting would be useful in surveillance of TB transmission and intervention to prevent further spread of this disease. The specificity and sensitivity of this technique has been found to be 98 and 96%, respectively with the clinical samples (Soolingen et al., 1995; Gori et al., 2005; Soolingen, 2008).

Restriction fragment length polymorphism (RFLP)

It is considered as a gold standard for the molecular typing of MTC due to its high discriminative power and reproducibility. It can also be used for outbreaks identification and can facilitate contact tracing of TB (Sharma and Gupta, 2011). However, this technique requires large amount of DNA and is therefore restricted to the mycobacterial cultures which take around 20 to 40 days to obtain sufficient DNA needed and for the combined process of probe labeling, DNA fragmentation, electrophoresis, blotting, hybridization, washing and autoradiograph. Moreover, this technique is also technically demanding, slow, cumbersome, expensive and requires sophisticated analysis software for result analysis (Patterson and Grooms, 2000; Sharma and Gupta, 2011).

Variable number tandem repeats typing (VNTR)

Genetic loci containing variable numbers of tandem repeats (VNTR) loci form are the basis for human gene mapping, forensic analysis, and paternity testing. In this technique, DNA containing VNTR sequences is amplified by PCR and the size of the product determined by gel electrophoresis identified six VNTR loci (ETR-A to F) for typing the MTC. When compared to the RFLP-IS6110 fingerprinting, VNTR was demonstrated to be less discriminatory for strains with a high copy number of IS6110, but allowed improved discrimination for strains with only one or two copies of IS6110. The usefulness of this technique has not yet been fully assessed for *M. bovis*, although its evaluation is underway for isolates from the Great Britain at the Veterinary Laboratories Agency. Preliminary results suggest that although VNTR gives a higher degree of discrimination than spoligotyping, best results are obtained by combining the two techniques (Sharma and Gupta, 2011).

Xpert MTC test

It is an automated molecular test for the diagnosis of MTC infections and the identification of antimicrobial multi-resistance using sputum samples (Helb et al., 2010). This test allows for considerable automation with all reagents used for bacterial disruption, nucleic acid extraction, amplification and amplicon detection inside a disposable cartridge. Results are generated within two hours of processing. The system has shown promising results. Amongst culture positive patients a test sensitivity was estimated of 98.2% in smear positive (microscopy)

patients and 72.5% in smear negative patients (Boehme et al., 2010). The test had a sensitivity of 90.3% as compared to 67.1% for microscopy in culture positive patients (Boehme et al., 2011). However, financial constraints may limit their widespread usefulness, as concerns have been expressed regarding the cost of the GeneXpert system (Kranzer, 2011). In Ethiopia, it has been used for diagnosis of multi-drug resistance, pediatric and HIV co-infected TB in clinical and research setting.

CONCLUSION

Conclusively, the advantages and disadvantages of each available TB diagnostic method are evident and no test is yet available to meet target specification in terms of performance and ease of use. Performance is a compilation of sensitivity, specificity and speed, while ease of use is a compilation of safety, number of steps, cost, robustness and training simplicity. The clinical, histological, radiology, microscopy and Xpert MTC test are used in clinical setting for diagnosing patient in Ethiopia. While other microbiological, immunological and molecular methods are widely used in research purpose. Thus, to overcome limitations of specificity and sensitivity of diagnostic techniques in clinical setting and introducing reliable techniques need attention for both clinical and research setting.

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Conflicts of interest

The authors declare no conflict of interest.

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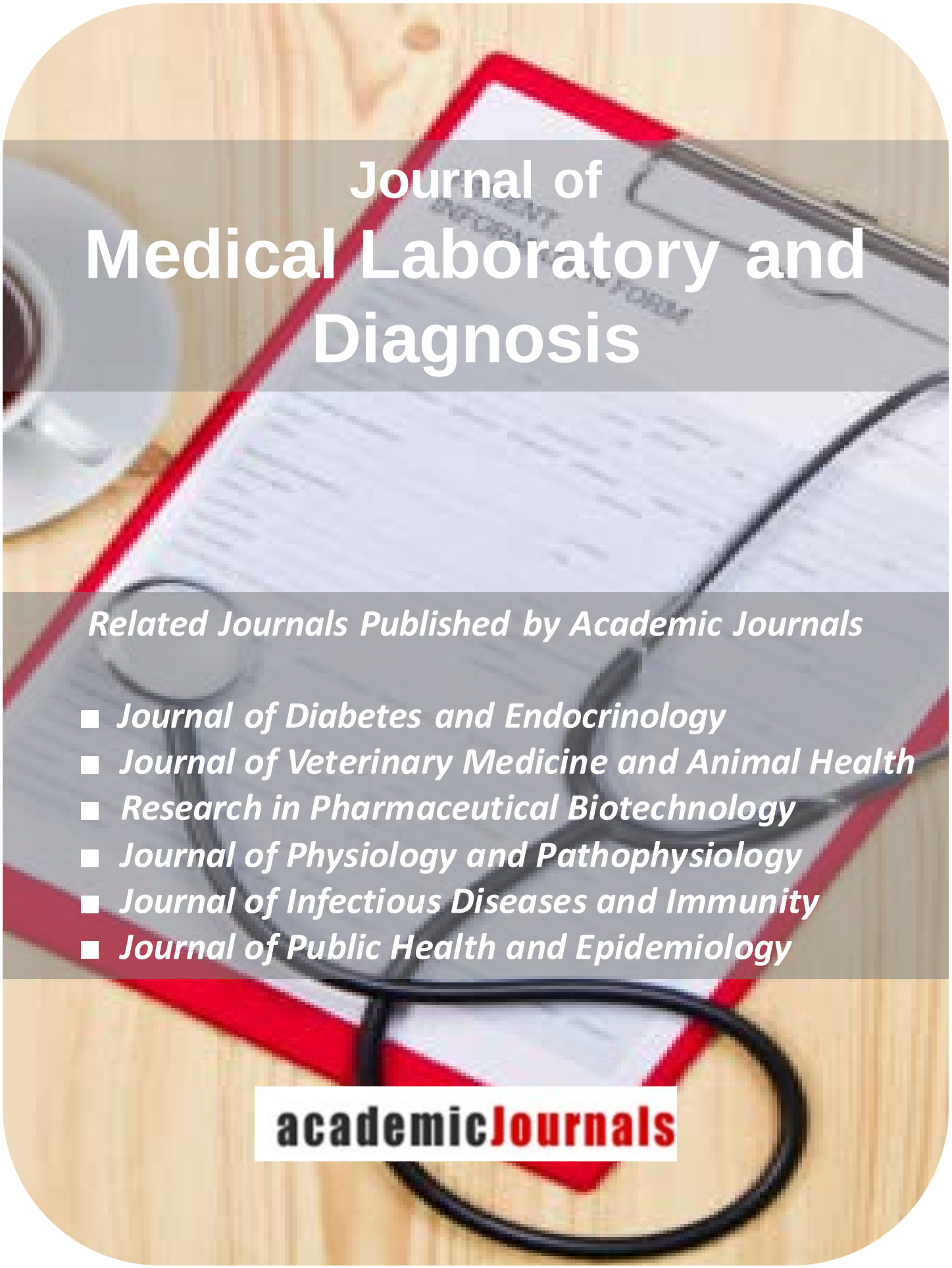

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