EVALUATION OF GENEXPERT MTB/RIF ASSAY FOR THE DIAGNOSIS OF CHILDHOOD PULMONARY TUBERCULOSIS FROM STOOL SPECIMEN AT JIMMA UNIVERSITY MEDICAL CENTER, SOUTHWEST, ETHIOPIA

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SCHOOL OF GRADUATE STUDIES

Evaluation of GeneXpert MTB/RIF Assay for the Diagnosis of Childhood Pulmonary Tuberculosis from Stool Specimen at Jimma University Medical Center, Southwest, Ethiopia

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ASTRACT

Background: Diagnosis of tuberculosis (TB) in children is challenging due to non-specific symptoms, difficulty of obtaining respiratory specimen and lack of sensitive diagnostic tests. The available TB diagnostic tests require sputum specimen, which is very difficult to obtain from young children. Other specimen like gastric aspirate (GA) can be applied to make microbiological diagnosis. However, these methods are complex, relatively invasive and often need the children to be hospitalized and overnight fasting. Thus, it needs alternative non-invasive specimens for diagnosis of pulmonary TB in children unable to produce sputum.

Objective: To evaluate the diagnostic performance of Xpert assay for diagnosis of TB from stool specimen in children <15 years with presumptive pulmonary TB.

Method: A cross-sectional study was conducted at Jimma University Medical Center from 1st March to 30th November 2019. Socio-demographic and clinical data of participants were collected using structured questionnaires. Expectorated sputum or GA specimens were collected and analyzed by Xpert assay and Lowenstein-Jensen (LJ) culture. In addition, one stool specimen was collected from each child and tested by Xpert assay. Based on clinical, radiological and laboratory findings, patients were categorized into four groups: “confirmed TB”, “probable TB”, “possible TB”, and “not TB”. Diagnostic performance of stool Xpert was calculated with reference to LJ culture and composite reference standards (CRS).

Results: A total of 178 children were enrolled, 169 had complete microbiological and clinical results. Male to female proportion was (81/88). The mean age was 3years, the majority 64.5% of the participant age was <5 years old. The overall microbiologically and clinically confirmed childhood TB proportion was 11.8% (20/169). Of these, 5.9% (10/169) had confirmed TB, 2.4% (4/169) had probable TB, 3.6% (6/169) had possible TB and 88.2% (149/169) had not-TB. Stool Xpert had sensitivity of 100% (95%CI: 66.4-100) and specificity of 99.3% (95%CI: 96.2-100) compared to culture. Whereas, the overall sensitivity and specificity of stool Xpert was 50% (95%CI: 27.2-72.8) and 100% (95%CI: 97.1-100) compared to CRS.

Conclusions: Stool Xpert testing was improved sensitivity than GA Xpert testing that can be easily implemented at lowest level of health care system. However, a negative Xpert assay results may not exclude a diagnosis of TB in children and children with strong clinical findings suggestive of TB should be started on ant-TB treatment.

Keywords: - Children, Pulmonary tuberculosis, Sensitivity, Specificity, Stool, Xpert MTB/RIF

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LIST OF ABBREVIATION/ACRONYMS

AFB	Acid Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
ATT	Anti-TB Treatment
BCG	Bacille Calmette-Guérin
BSC	Biological safety cabinet
CFU	Colony forming unit
CRS	Composite Reference Standard
CT	Cycle threshes hold
CXR	Chest X-ray
DNA	Deoxyribonucleic acid
DR-TB	Drug Resistant Tuberculosis
DST	Drug Susceptibility Test
EPTB	Extra Pulmonary Tuberculosis
FIND	Foundation for Innovative New Diagnostics
FM	Fluorescence Microscopy
GA	Gastric aspirate
HIV	Human Immunodeficiency Virus
IS	Induced sputum
JU	Jimma University
JUMC	Jimma University Medical Center
LED	Light-Emitting Diode
LJ	Lowenstein-Jensen
LTBI	Latent Tuberculosis Infection
MDR-TB	Multidrug Resistant Tuberculosis
MRC	Mycobacteriology Research Center
MTBC	Mycobacterium tuberculosis Complex
NAAT	Nucleic Acid Amplification Test
NaOH-NALC	Sodium Hydroxide -N-Acetyl -L-Cystein
NPV	Negative Predictive Value
NTM	Non tuberculosis Mycobacterium
PNB	para-nitro benzoic acid
PBS	Phosphate Buffered Solution

PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
PTB	Pulmonary Tuberculosis
RIF	Rifampicin
RIF-R	Rifampicin resistance
SOP	Standard Operating Procedures
SPSS	Statistical Package for the Social Science
ST	String test
TB	Tuberculosis
TST	Tuberculin skin testing
USNIH	United States National Institutes of Health
WHO	World Health Organization
XDR-TB	Extensively Drug Resistant Tuberculosis
ZN	Ziehl-Neelsen

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1. INTRODUCTION

1.1. History of Tuberculosis

Humans have suffered from TB since ancient time and TB has been described in ancient texts from China about 2,300 years ago (1). Fragments of the genome of *M. tuberculosis* the causative agent of TB have been detected in 3,300-year-old Egyptian mummy (2). The TB epidemic reached a peak in Europe and North America in the 18th and 19th century, and scientists started to focus more on TB pathogenesis (1). Robert Koch discovered that *M. tuberculosis* is the actual infectious agent of the disease. His announcement of this seminal discovery on 24th March 1882 is still celebrated as the World TB day. He received the Nobel Prize in Medicine for this discovery in 1905; the bacterium is also known as "Koch's bacillus. Subsequently, Paul Ehrlich developed acid fast staining method to detect *M. tuberculosis* which was later improved by Ziehl and Neelsen (3).

Historically, childhood TB has been neglected in global TB community and the health community in general due to difficulties in confirmation of a TB diagnosis in children, misperception of childhood TB as a low public health priority, a misplaced reliance on the protective efficacy of the Bacillus Calmette-Guerin (BCG) vaccine and the lack of systematic data collection on childhood TB (4).

1.2. Etiology of Tuberculosis

TB is an airborne disease caused by the bacterium *M. tuberculosis* which belongs to the group of *M. tuberculosis* complex (MTBC) within the family of Mycobacteriaceae (5). The group of MTBC comprises seven very closely related mycobacterial species (*M. bovis, M. africanum, M. microti, M. caprae, M. pinnipedii, M. canetti* and *M. mungi*). Not all of these species have been found to cause disease in humans. The majority of TB cases are caused by *M. tuberculosis*. Mycobacteria are aerobic, non-motile and non-sporulated bacilli with a relatively slow replication rate compared to other bacteria (about 24 hours per generation). It possesses a unique cell wall structure (6), which is rich in unusual lipids, glycolipids and polysaccharides (7). The mycolic acid, the major lipid compound in the cell wall, is strongly hydrophobic, allowing the bacteria to survive in the macrophages and resist hydrophilic drugs and dehydration (8).

1.3. Transmission and Pathogenesis of Tuberculosis

Transmission of TB occurs when a person inhales droplet nuclei containing *M. tuberculosis*, and the droplet nuclei traverse the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lungs. *M. tuberculosis* is carried in airborne particles, called droplet nuclei, of 1–5 microns in diameter. Infectious droplet nuclei are generated when persons who have PTB disease cough, sneeze, shout, or sing. Depending on the environment, these tiny particles can remain suspended in the air for several hours (9).

Infection occurs when a person inhales droplet nuclei containing tubercle bacilli that reach the alveoli of the lung. These tubercle bacilli are ingested by alveolar macrophages and subsequently, the localized inflammatory response occurs, recruiting mononuclear and other cells of the immune system from the surrounding blood vessels. There are three possible outcomes to the bacilli within the macrophage: (i) the majority of these bacilli are eliminated or cleared by the host immune response, or (ii) the infection may progress to active TB disease when the pathogen multiply inside the macrophages and released in to lymphatic channels (iii) become dormant or latent TB infection (LTBI) when an equilibrium established between the host immune response and the pathogens (9). The progress from LTBI to active TB disease can occur at anytime in person's life depending on age and immune status of the host. In most cases less than 10%, of persons infected with TB develops sign and symptoms of active TB disease over a lifetime (10).

1.4. Risk Factors of Tuberculosis

The risk of TB infection in children is usually higher than those for adults in high burden countries. The major risk for them lies in the infected adult they are in contact with. Other risk factors include severe malnutrition, exposure to smoke, having a female index case, the use of immunosuppressive drugs, lack of BCG vaccination, overcrowding, poor treatment for previous TB disease, diabetes and poverty are the major risk factors for TB (11). The risk of developing disease after infection is determined by various factors, including age at exposure, nutritional and immune status, virulence of the organism, and magnitude of initial infection (12).

1.5. Clinical Features of Tuberculosis in Children

TB can affect any organ in the body but the commonest type of TB in children is PTB. Children often have an atypical clinical presentation, but in children with suspicion of PTB the usual presenting symptoms are a persistent, non-remitting cough or wheeze that is

unresponsive to the treatment for an alternative cause (e.g. bacterial pneumonia), fever which does not improve after anti-malarial treatment and weight loss (13).

1.6. Diagnosis of Childhood Tuberculosis

The accurate and prompt diagnosis of TB in children is essential, as delayed or incorrect diagnosis may lead to disseminated disease, death or severe squeal in children. The diagnosis of TB in endemic countries is based on history, physical examination, contact investigation and the results of available diagnostic tools (14). Smear microscopy is usually available but it requires high bacterial loads in clinical specimens for detection of AFB, however childhood TB is often smear-negative due to the presence of low bacilli load in clinical specimen. *M. tuberculosis* culture is rarely available in endemic countries outside national reference laboratories. Hence, the diagnosis of TB in children is more challenging compared to adults for several reasons: due to non-specific clinical symptoms, difficulty of obtaining quality specimen required by conventional diagnostic tests and difficulty of confirming in microbiological diagnostic tests due to the presence of low bacillary load in the clinical specimen of children (15).

1.7. Statement of the Problem

TB remains a major health problem among millions of children each year worldwide. It is the leading cause of death from a single infectious agent. In 2018, an estimated 1.1 million (11%) of children (less than 15 years) developed TB worldwide. In the same year, there were an estimated 205,000 children died due to TB disease globally. The 30 high burden countries (HBC) for TB accounted 87% of new TB cases. Majority of TB cases occurred in children were in south-east Asia and African countries. Ethiopia is one of the 30 HBC for TB in the world and TB is an important cause of illness and death in children in the country (16).

Diagnosis of TB in children is challenging due to non-specific clinical presentations, difficulty of obtaining respiratory specimen and lack of sensitive diagnostic tests (15). Sputum specimen remains the most important clinical samples used for current microbiological test to confirm TB (17). The yield is expected to be suboptimal for children with PTB who are unable to expectorate sputum specimen. As an alternative, different specimen types have been studied to improve the sensitivity of microbiological examination, such as induced sputum, bronchoalveolar lavage, and gastric aspirate (18). However, the collection of such type of specimens is resource intensive and relatively unsafe for children.

Stool is an alternative specimen for TB tests, because *M. tuberculosis* can be swallowed and detected in the samples from digestive tract (19). In particular, stool is easy to obtain from young children unable to produce sputum, the diagnosis of whom in more challenging (20). WHO endorsed the automated nucleic acid amplification test (NAAT) Xpert MTB/RIF assay for the diagnosis of *M. tuberculosis* from various specimen types (21). In Ethiopia, there is a paucity of data on the diagnostic performance of Xpert MTB RIF assay on stool specimens for children unable to expectorate sputum. Therefore, we evaluated the diagnostic performance of Xpert MTB/RIF assay for diagnosis of TB from stool specimens in children with presumptive PTB.

1.8. Significance of the Study

TB is an important cause of morbidity and mortality in children in Ethiopia. However, the diagnostic evaluation of Xpert MTB/RIF assay in stool specimen of children with presumptive PTB is limited. The finding from this study will be used to fill the information gap on the diagnosis of childhood PTB from stool specimen by Xpert MTB/RIF assay. Help the clinicians to improve TB diagnosis and management. Serve as one important input for Ethiopian Health planners, policy makers and care providers for designing diagnosis, control and prevention strategy among the study group. It will be used as a growing source of evidence for the diagnosis of childhood PTB. Serve as a reference document for researchers to embark on studies of the same or related kinds in other parts of the country.

2. LITERATURE REVIEW

2.1. Global Burden of Tuberculosis

TB remains a major global health problem, despite efforts and interventions for several decades. It is the leading causes of death from a single infectious agent worldwide. In 2018, there were 10 million new TB cases, of which 57% cases were among men, 32% among women and 11% among children. It was estimated that 1.2 million people died from TB among HIV-negative people and an additional 251,000 deaths from TB among HIV-positive people in 2018, of these 55% of deaths among HIV-negative people were men, 31% women and 14% children (aged <15 years) and an additional 49% of deaths among HIV-positive people were men, 38% women and 13% children. About 85% of TB deaths in both HIV-negative and HIV-positive peoples occurred in African and South-East Asian countries (16).

Most of the estimated number of TB cases occurred in Asia (44%), Africa (24%) and Western Pacific (18%); smaller proportions of cases occurred in the Eastern Mediterranean (8.1%), the European Region (2.6%) and America (2.9%). The 30 HBC accounted for 87% of all estimated incident cases worldwide, and eight of these countries accounted for two thirds of the global total: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (6%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). An estimated 8.6% (range, 7.4–10%) of the incident TB cases in 2018 were among people living with HIV. The proportion of TB cases co-infected with HIV was highest in countries in the African country, and exceeded 50% in parts of Southern Africa (16).Today global TB control is facing major challenges. It still requires much effort to ensure quality care accessibility without barriers of gender, age, type of disease, social setting, and ability to pay (12). Co-infection of TB/HIV, multi-drug resistant (MDR) and extensively drug resistant (XDR) TB in all regions, especially in Africa make control activities more complex and demanding (22).

2.2. The Burden of Tuberculosis in Ethiopia

TB is among the leading causes of morbidity and mortality in Ethiopia. Currently, an estimated 151 per 100,000 populations of incident TB cases occurred in the country, ranking 10th among the world's 30 HBC for TB, and 4th in Sub-Saharan Africa. While TB kills an estimated 22,000 Ethiopians per year, it has a long-term impact on the economy of the country. The majority of TB cases occur among adolescents and children, cumulatively exerting a heavy economic cost, hampering the country's drive toward becoming a middle-income country (16). Children can usually be infected with TB by an adult or an older child

with sputum smear-positive PTB. They may also be infected by contact with smear-negative but culture-positive cases less commonly. Childhood TB is also a marker of TB transmission within a community (23). Study conducted in Tigray, Ethiopia revealed that 8.1% (1086/13,345) of TB cases were occurred in children <15 years old (24). Other study conducted in North western part of Ethiopia, reported that 22% (484/2240) of incident cases of pediatric TB occurred in children aged less than 5years (25).

2.3. Diagnostic Methods of Active Tuberculosis

Early detection of the cases and prompt treatment are crucial for TB control. TB diagnosis mainly depends on the clinical presentation of the disease and identification of the bacilli in clinical samples. Many TB diagnostic tests are available although no single diagnostic test for TB exists that can be performed rapidly, simply, inexpensively, and accurately. Some of the widely used methods for detection of active TB are: microscopy, nucleic acid amplification tests (NAATs), and cultures (26).

2.3.1. Smear Microscopy

Smear microscopy is the oldest and still the most widely used methods for diagnosis of TB in low-resource settings (27). The principle of the techniques is based on the fact that the cell wall of mycobacteria is rich in complex lipids that prevent access to gram staining, but when stained with carbol-fuchsin or fluorochromes under special staining conditions, these are not easily decolorized, even with alcohol-acid solutions and referred to as acid fast bacilli (AFB). There are two types of acid fast stains are used to detect mycobacteria in clinical specimens: (i) Ziehl-Neelsen (ZN) stain, the bacilli stained by this method appear as red rods. (ii) Fluorochrome (auramine or auramine-rhodamine) staining, in this method, the dye facilitate the observation of bright fluorescent AFB against a darker background under fluorescence microscopy (FM). This method is on average 10% more sensitive than ZN stain methods and permits more rapid screening (at lower magnification) of large numbers of smears. However, fluorochrome staining gained only 2% of clinical sensitivity compared to ZN staining for diagnosing TB in children (14.6% versus 12.5%, (28).

Smear microscopy is simple, inexpensive and efficient in detecting those cases of PTB that is most infectious. A major limitation of smear microscopy is its low sensitivity (25–75%) compared to culture for diagnosis of PTB in adult patients and the high number of bacilli required for positivity with estimated detection limit of between 5,000–10,000 bacilli/ml of clinical sample to be visually detected (10). Moreover, the sensitivity of smear microscopy is

poor particularly in childhood TB (29). In a recent review, the pooled value of sensitivity of ZN staining was found to be less than 10% on sputa (30), and very poor on GA in children. The inefficiency of smear microscopy in children could be explained by the paucibacillary nature of pediatric TB (31).

2.3.2. Mycobacterial Culture

Culture is the WHO-recommended gold standard test for the diagnosis of TB disease. However, the sensitivity of culture for detection of *M. tuberculosis* for children is much lower than that for adults due to the paucibacillary nature of the clinical specimen (15). Culture of *M. tuberculosis* is performed on agar based medium (solid culture) or liquid medium (liquid culture). The commonly used solid media are the egg-based Lowenstein-Jensen (LJ) or agar-based Middlebrook 7H10 media. Liquid culture is more sensitive for MTBC detection and also has shorter time to positivity, however it is also more often contaminated, or cross contaminated.

In microbiology laboratories in resource-limited countries, LJ slants are the most commonly used solid medium because of lower cost and being easy-to-use (32). The major limitation of this method is the slow turnaround time of 4-6 weeks, which is too late to influence clinical decision-making. In addition, LJ has a lower yield in comparison to liquid culture in diagnosing TB (33). The general limitation of culture is it requires technical skills and a high complex laboratory facility that cannot be performed at lower health care facility, and require more time for drug susceptibly testing (10).

2.3.3. Xpert MTB/RIF assay

Xpert MTB/RIF is a molecular cartridge-based NAAT developed by United States National Institutes of Health (USNIH) and Foundation for Innovative New Diagnostics (FIND) partnered with Cepheid (Sunnyvale, California, United States) and the University of Medicine and Dentistry of New Jersey, which can simultaneously detect MTBC and rifampicin resistance (RIF-R) (34). The test has accomplished by automating most of the steps required to process clinical samples. It uses hemi-nested real-time polymerase chain reaction (RT-PCR) that utilizes molecular beacon technology to detect deoxyribonucleic acid (DNA) of MTBC using three specific primers and five unique molecular probes.

The assay provides results with less than 2 hours, and first endorsed by WHO in 2010 and it was recommended for TB diagnosis in children since 2013 (21). However, the diagnostic performance of Xpert MTRIF assay for diagnosis of TB in children is poor compared to adult

patients due to low bacillary loads in clinical specimen of children. The overall, estimated sensitivity of Xpert MTB/RIF assay for children is 62-66% in expectorated or induced sputum (IS) and GA specimens, respectively when compared with culture as reference method (35).

2.4. Specimen Collections

Alternative less invasive respiratory specimens have been studied to facilitate specimen collection and diagnostics in children. The specimens including IS which is collected using hypertonic saline to irritate the airways to induce cough and obtain material from lung airways (36). Sputum induction is unpleasant for patients, but was reported to be safe and useful for MTB confirmation of PTB in both HIV-infected and HIV uninfected children (37). However, the culture detection rate of IS was found to be less than of GA (17.9% versus 32.5%) for diagnosing TB among children (38). The string test (ST), which is a method to retrieve enteric pathogens, is a potential tool for detection of MTB in patients who are unable to produce sputum (39). The ST uses an absorbent nylon string coiled inside a gelatin capsule. The culture yield of ST for diagnosis of TB in children was also reported to be comparable to IS in the study of evaluating different specimen types for diagnosis of TB in children (40). However, the major disadvantage of ST is that it is not always well tolerated by young children (41).

Recently, some attention has been focused on investigating other types of specimens which are easily collected such as stool and urine (42),(43). Detecting MTB by Xpert MTB/RIF assay on stool samples from children having PTB was 47.1% sensitive as compared to the sputum MGIT culture standard (43). In a recent small study using a modified stool processing protocol, the sensitivity of Xpert MTB/RIF on stool was 85% of positive cases by Xpert MTB/RIF on IS or gastric aspirate (44). However, stool culture for mycobacterial detection in children with PTB had low sensitivity and higher contamination rates (45). Collection of multiple samples has been frequently reported to increase the overall yield of detection of pediatric TB cases compared to single specimens (46). The detection rate of culture by two consecutive gastric aspirates was 67% versus 38% by single sample in diagnosing PTB among children in Cape Town, South Africa (47). Combination of different specimen types also was found to be a potential approach to improve the diagnosis TB in children (48).

3. OBJECTIVES OF THE STUDY

3.1. General Objective

- To evaluate the diagnostic performance of Xpert MTB/RIF assay from stool specimen in children with presumptive pulmonary tuberculosis at Jimma University Medical Center, Southwest Ethiopia.

3.2. Specific Objectives

- To determine the proportion of microbiological confirmed TB among children with presumptive PTB.
- To determine the diagnostic performance of Xpert MTB/RIF assay in detecting TB from stool specimens among children with presumptive PTB.
- To compare the yield of MTBC from stool versus GA specimens in Xpert MTB/RIF testing among children with presumptive PTB.

4. MATERIALS AND METHODS

4.1. Study Areas and Periods

The study was conducted at Jimma University Medical Center (JUMC), Southwest direction of Ethiopia from 1st March to 30th November 2019. JUMC is one of the oldest public hospitals in the country. It is located in Jimma city 352 km southwest of Addis Ababa, capital city of Ethiopia. Currently, it is the only teaching and referral hospital in the southwestern part of the country, providing services for approximately 15,000 inpatient, 160,000 outpatient attendants, 11,000 emergency cases and 4500 deliveries per year from a catchment population of about 15 million people (49).

Laboratory investigation was carried out at Jimma University Mycobacteriology Research Center (JUMRC). The center's activities are mainly focused on basic research, training and service in the field of Mycobacteriology.

4.2. Study Design

An institution based prospective cross-sectional study was conducted.

4.3. Populations

4.3.1. Source Population

All children less than 15 years of age who visited JUMC during the study period

4.3.2. Study Population

Children less than 15 years of age who were suspected of having PTB by the treating clinicians during the study period

4.4. Eligibility Criteria

4.4.1. Inclusion Criteria

Children <15 years old, whose parents/care givers were willing to participate and who fulfill at least one of the listed criteria were included in the study:

- Presenting symptoms of persistent, non-remitting cough for ≥2 weeks, unexplained fever (>38^0C)/night sweet for ≥1 weeks, weight loss, fatigue, reduced playfulness, decreased activity and poor appetite.
- History of contact with index TB cases (children contact with a known case of TB in house hold and other close contact for the past 12 months).

4.4.2. Exclusion Criteria

Children ≥15 years old, patients who were initiated on anti-TB treatment prior to collecting the clinical specimen, severely ill patients who were unable to provide the clinical specimen.

4.5. Sample Size Determination and Sampling Technique

4.5.1. Sample Size Determination

The sample size required for this study was calculated using formula for estimating sample sizes for evaluating sensitivity and specificity of diagnostics test (50). To calculate the sample size, 96% sensitivity of Xpert MTB/RIF from previous study (51), with absolute precision of 5% at 95% confidence interval (CI) was used. Prevalence of PTB of 31.7% was used (52). With this given data, 187 study participants who fulfill the inclusion criterion were eligible. Sample size was calculated by the following formula:

$$\text{Sample size (n) based on sensitivity } n = \frac{Z^2_{1-a/2} \times S_N \times (1-S_N)}{d^2 \times P}$$

$$= \frac{(1.96)^2 \times 0.96 \times (1-0.96)}{(0.05)^2 \times 0.317}$$

$$= \frac{0.14751744}{0.0007925}$$

$$n=\mathbf{187}$$

Where:

n = required sample size

S_N = anticipated sensitivity of Xpert MTB/RIF assay from previous study (96%)

α = size of the critical region ($1 - \alpha$ is the confidence level)

$Z^2_{1-\alpha/2}$ = standard normal deviate that corresponds to 95% confidence interval (1.96) and

d = absolute precision (d=5%)

P= estimated prevalence of presumptive pediatric PTB in the study area from previous study (31.7%)

Note: the available participants during the study period were 178 we were not able to add more participants due to time and resources constraints.

4.5.2. Sampling Techniques

Non-probability, consecutive sampling technique was used.

4.6. Data Collection Procedures

Patients who visited JUMC from the different areas of Southwest, Ethiopia for routine clinical care were our source populations. Upon arrival, all patients were clinically examined and those who were suspected of having PTB by the clinicians that fulfill the inclusion criteria and willing to participate in the study were consecutively enrolled after having written informed consent of the children parents and those aged 10 and above years having signed assent by the children. Demographic data and medical history of study participants were collected through structured questionnaires. Patient's medical records were examined for a clinical diagnosis of TB. Clinical data of radiology findings, clinical improvement after anti-TB treatment (ATT), HIV status, nutritional status, vaccination status, antibiotic treatment and the final diagnosis made by the clinicians for the patients were collected from their medical records. One respiratory and stool specimen was collected per child. The respiratory specimens were divided in to two parts. One part of the specimen was tested by Xpert MTB/RIF assay and the second part of the specimen was transported to JU-MRC for culture. The minimum acceptable volume of respiratory specimen was 4ml for sample processing. Acidic GA specimens were neutralized by phosphate buffered solution (PBS) pH 6.8 before processing for culture and stored at 2-8^{0}C for 2-3 days until processed for culture. Stool specimens were also transported to MRC laboratory and stored at -20°C until tested by Xpert MTB/RIF assay.

Based on the results of Xpert MTB/RIF assay, solid culture, radiological finding and clinical response after ATT, the patients were categorized into four groups according to previous study (53), Confirmed TB, Probable TB, possible TB, not TB.

4.7. Operational Definitions

Presumptive PTB: children with the presenting sign and symptoms suggestive of PTB

Composite reference standard: defined as more than two tests are used to evaluate the diagnostic performance of the index test in the absence of single perfect reference standard, in this study the CRS we used was Xpert MTB/RIF assay, culture and clinical improvement after ATT.

Microbiologically Confirmed TB: is one from whom a respiratory specimen tests positive by Xpert MTB/RIF assay or LJ culture.

Probable TB: refers to cases in which microbiological confirmations were not obtained, but radiological findings suggestive of TB and clinical improvement after ATT.

Possible TB: refers to cases in which microbiological confirmations were not obtained, and radiological findings were not suggestive of TB, but clinical improvement after ATT and has no alternative diagnosis available.

Not TB: refers to cases which do not fulfill the criteria set for confirmed, probable and possible TB case, but alternative diagnoses were available.

Clinically diagnosed TB: refers to cases which do not fulfill the criteria set for microbiological confirmations, but has been diagnosed with active TB by the clinicians who have decided to give the patient a full course of ATT.

Clinical improvement: clinical features suggestive of TB disease that were present at baseline have improved after ATT initiation.

CXR findings; includes Hilar/mediastinal lymphadenopathy, miliary infiltrate, Cavitation, Pleural or pericardial effusion. CXR was classified as consistent with PTB, if there is a positive response for any one of the above radiologic features.

Sensitivity: The ability of Xpert MTB/RIF assay to identify correctly those who have the TB disease from the suspected patients.

Specificity: The ability of Xpert MTB/RIF assay to identify correctly those who do not have TB disease from the suspected patients.

PPV: The proportion of patients who test positive who actually have the disease

NPV: The proportion of patients who test negative who are actually free of the disease

4.8. Laboratory Investigations

Xpert MTB/RIF assay and solid egg based LJ medium for TB culture was used. Xpert MTB/RIF assay was performed at JUMC laboratory from respiratory specimens, whereas TB culture and stool Xpert test were performed at JUMRC. The overall sample collection and processing workflow is described blow in (**Figure 1**).

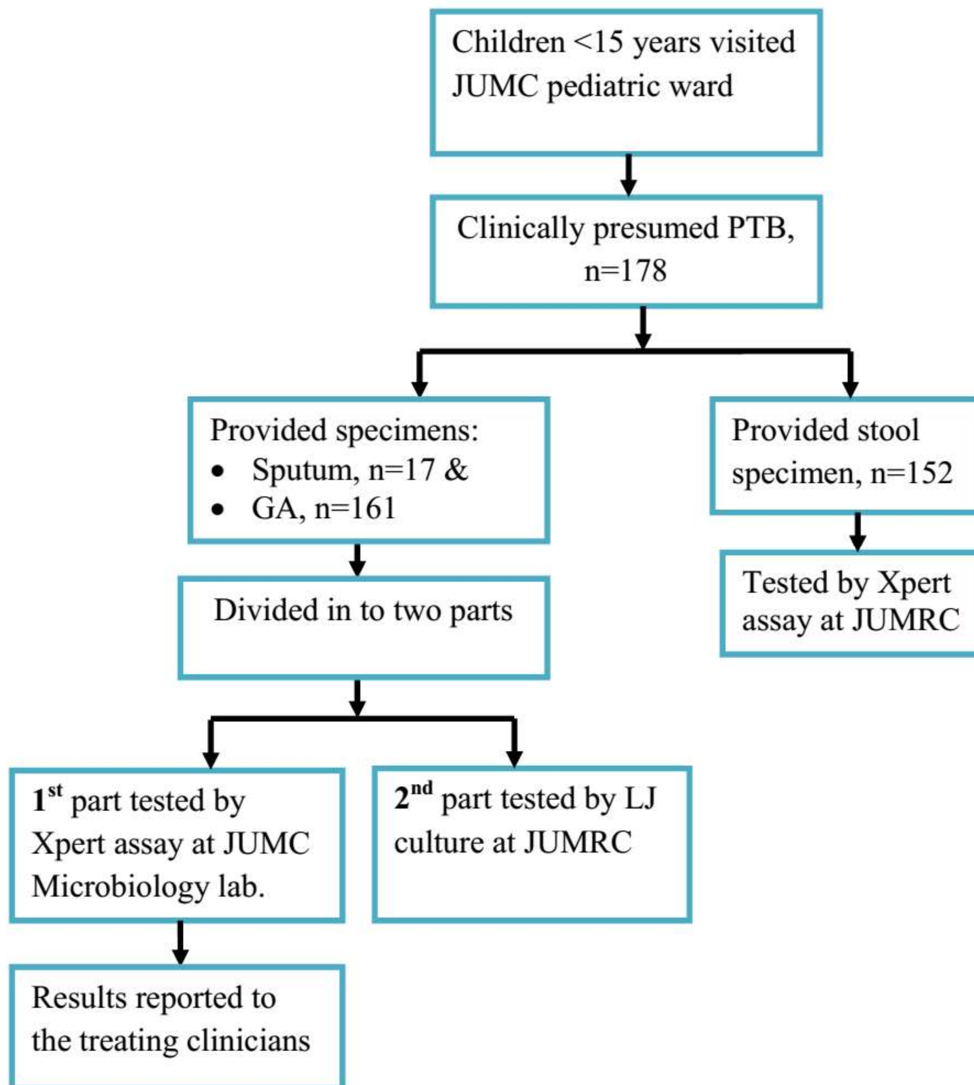

Figure 1: Diagrammatic Representation of the Study Workflow

The early morning expectorated sputum specimens were collected by patients in labelled sterile falcon tube after the patients were explained the importance of submitting thick sputum by the clinicians. It was demonstrated that washing their mouth with clean water, followed by taking three deep breaths and a deep cough, a good quality sputum could be brought from the lungs, opening the cup and holding it close to their mouth spit the mucous into the cup without getting any on the outside of the cup and then screw the lid on tightly to prevent leakage. Then, the specimens were sent to JUMC microbiology laboratory with the patients request forms. Upon arrival of the sputum specimen to the laboratory the quality of the sputum was checked by the laboratory professionals before processing the specimen. GA specimens were collected early morning after overnight fasting by trained nursing staff using

nasogastric tubes connected with sterile syringe and the specimens were sent to JUMC microbiology laboratory with the patients request form. Upon arrival of the specimen in the laboratory, respiratory specimens were divided into two parts by laboratory personnel. The first part of the specimen was processed and tested by Xpert MTB/RIF assay at JUMC microbiology laboratory and the second part of the specimen was transported to JUMRC and acidic GA specimens were neutralized by PBS pH6.8 at the time of collection with equal volume of the specimen and stored at 2-8^{0}C until processed for culture within 2-3 days of collection.

4.8.1. Respiratory Specimen Processing for Xpert MTB/RIF assay

The first part of respiratory specimen was diluted (2:1 v/v) with Xpert MTB/RIF sample reagent followed by shaken vigorously and incubated for 15min at room temperature. The liquefied specimen was aspirated until the meniscus is above the minimum mark by sterile transfer pipette provided with Xpert MTB/RIF kit and transferred to open port of Xpert MTB/RIF cartridges and loaded in to GeneXpert instrument to run the test. The results were reported to the physicians.

4.8.2. Stool Sample Collection and Transportations

Approximately, 3gm of stool specimen was collected from each patient in sterile container (25ml fecal cup with spoon) labelled with patient's laboratory code and transported to JUMRC and stored at -20^{0}C until tested by Xpert MTB/RIF assay.

4.8.3. Stool Specimen Processing

Stool specimen processing was done according to a simple stool testing protocol described by researchers at the KNCV TB Foundation (54). Stored raw stool specimens (-20°C) were thawed at room temperature. Approximately, 1gm of stool specimen was transferred to a sterile 50ml falcon tube using applicator stick and 8ml of sample reagent buffer was added to sample tube and mixed very well. The tube was kept for 20min at room temperature to gravitate solid particles. Then, 2ml of the supernatants were carefully aspirated by a sterile pipette and transferred into the open port of pre-labelled Xpert MTB/RIF cartridge. The prepared cartridge was loaded into GeneXpert diagnostic instrument and allowed to start the Xpert MTB/RIF assay. Then, the results were reported by Xpert MTB/RIF assay within 2hrs after the test was run.

4.8.4. Xpert MTB/RIF assay Validity Experiment

Validity check was performed before stool Xpert test was done as indicated below: -

A total of five stool specimens were spiked with the *M. tuberculosis* H37Rv reference strain. Briefly, H37Rv was cultured on LJ medium. Using a sterile wire loop, 3–5 well-isolated colonies were scrapped from the surface of LJ medium and transferred to cryovial tube containing 2ml of sterile distilled water and emulsified it with sterile glass beads. Then, the suspension was heat killed in water bath at 85^0c for 60min. After heat killed using sterile micropipette, 1ml of the supernatant was aspirated and transferred to sterile tube containing 2ml of distilled water which was used for matching as McFarland standard. The bacterial suspension's turbidity was matched with that of commercially prepared McFarland standard No 1. This suspension was prepared for 10^{-4} serial dilution. Four tubes containing 2ml of distilled water was prepared and labelled as dilution one (D1), dilution two (D2), dilution three (D3) and dilution four (D4). Then, using sterile micropipette 2ml of the work suspension which was matched with McFarland standard No 1.0 was transferred to D1 and briefly mixed by vortex mixer. Again 2ml of suspension from D1 was transferred to D2 followed by vortex mixing. Also 2ml of suspension from D2 was transferred to D3 followed by vortex mixing and the same procedure was followed for D4.

Using sterile wooden applicator stick approximately, 1gm of stool specimen was transferred to five sterile falcon tube labelled as MF 1.0, D1, D2, D3 and D4. The stool specimen was spiked with 1ml of each suspension prepared. Then, 8ml of sample reagent was added in to the tube and mixed vigorously. The specimen tube was kept for 20min at room temperature. Using sterile pipette provided by the kit 2ml of the supernatant was carefully aspirated and transferred in to the open port of pre-labelled Xpert MTB/RIF cartridge followed by loading into GeneXpert instrument and allowed the test to run according to the manufacturer manual.

4.8.5. Validation Results

The stool specimens tested by Xpert MTB/RIF assay for this experiment were positive for *M. tuberculosis* with different cycle threshes hold (CT) value see **(Table1)**.

Table 1: Stool Xpert Validity Check Experiment Results

Specimen ID	Results	MTB- load	Highest Ct-value recorded
MF1.0	MTB-DETECTED	HIGH	17.3
D1	MTB-DETECTED	MEDIUM	18.6
D2	MTB-DETECTED	MEDIUM	20.7
D3	MTB-DETECTED	MEDIUM	21.6
D4	MTB-DETECTED	LOW	25.6

D=dilution, MF= matched with McFarland standard No 1.0, MTB=mycobacterium tuberculosis

4.8.6. Respiratory Specimen Processing for Culture

Upon arrival of respiratory specimen to the JUMRC laboratory, laboratory identification number for culture and all relevant specimen information's were recorded onto log book. Mycobacterial culture was done on LJ medium. Appropriate personal protective equipment and Biological safety cabinet (BSC) was used when handling and processing specimen in the laboratory. Workbench and cabinet surface was cleaned and sterilized properly before and after specimen handling and all biohazardous wastes were properly disposed off. Reagents were freshly prepared, replaced regularly and only one specimen container was open at any given time to limit cross contamination.

To each specimen that was received by 50ml conical centrifuge falcon tube, an equal volume of NALC-NaOH solution was added to the specimen tube. After mixing on the vortex, the sample tube was left standing for 20min at room temperature for liquefaction and decontamination. Then, sterile PBS pH6.8 was added up to 45ml to neutralize the alkaline solution. Next step was to concentrate by centrifugation at 3000g for 15min. After discarding the supernatant, the pellet was resuspended in 2ml PBS. The resulting diluted deposit was used as for inoculation into on the surface of LJ medium. One positive control *M. tuberculosis* H37Rv reference strain (ATCC 27294) and one negative control sterile PBS was inoculated to each LJ medium and processed with every batch during culture for quality control.

After aseptic removal of the condensed moisture observed at the bottom of LJ tube, 100µl of processed specimen suspension were inoculated on each slope of LJ slant. The tube was then incubated at 35-37 °C with cap loosened in a slanted position for 48-72 hours for an even distribution and absorption of the inoculums. After that, contamination was checked and the tube cap was tightened and placed upright in racks for the remaining incubation time. The culture tube was examined weekly on Monday for bacterial growth. The positive growth of MTBC was often detected within 3-6 weeks with the observation of rough, crumbly, waxy and non-pigmented colonies. However, if bacterial growth was observed within 5 days of incubation, rapidly growing mycobacteria were suspected. The culture is recorded as negative, if no growth was observed after eight weeks.

Definite diagnosis of TB was made by identifying MTBC organisms from a clinical specimen after growth was observed on the surface of LJ medium. In the current study phenotypic identification of MTBC was performed by using a para-nitro benzoic acid (PNB) test. The

test was performed with the host laboratory protocol indicated as: - 0.5gm of PNB powder was measured and dissolved in 10ml of dimethylformamide (DMSO). Then, 6ml of the solution was added into 600ml of LJ fluid and the solutions were mixed thoroughly before dispensed in tube. After the solutions were briefly mixed they were dispensed in the tube and inspissated at 85^0C for 50min. The complete batches of media were incubated at 37°C for 48 hours to monitor quality of the media. From the growth observed on the surface of LJ culture medium, using a sterile wire loop, 3–5 well-isolated colonies were scrapped from the surface of LJ medium and transferred to cryovial tube containing 2ml of sterile distilled water and emulsified it with sterile glass beads. Then, kept for 5min to allow large clump settled down. Using sterile micropipette 1ml of the supernatant was transferred in to another sterile tube containing 2ml of distilled water and the suspension was matched with commercially prepared McFarland standard No 1.0. This bacterial suspension was used as the work suspension and about 100µl of the work suspensions were inoculated on the surface of LJ medium with PNB and the control LJ medium free from PNB and incubated at $35\text{-}37^0$C. The result was recorded after 4weeks of incubation. Finally, our result indicated that there were no growths observed on LJ media containing PNB powder, while growths were observed on the control LJ media free from PNB powder, thus all the isolates were identified as MTBC.

4.9. Data Quality Management

Data collectors were trained prior to data collection. Data completeness was checked during data collection. Study participants were instructed prior to clinical specimen collection. Standard operating procedures (SOP) of the host laboratory were followed during laboratory analysis. To avoid subjective interpretation of test results the laboratory personnel were blinded to the results of the other test. The control strain H37Rv was also used to check the quality of LJ media. The expiration date for Xpert cartridges and reagents were checked prior to sample processing for quality control. The overall proportion of microbiologically and clinically confirmed TB cases was calculated based on the results of respiratory specimen culture, Xpert test and clinical improvement after ATT. The diagnostic performance of stool Xpert and the proportion was calculated for the patients who provide both respiratory and stool specimens and who have valid results of respiratory specimen culture, Xpert test and clinical improvement after ATT.

4.10. Data Processing and Analysis

Data were coded and entered to Epidata version 3.1 and exported to SPSS version 21 for analysis. Sensitivity, specificity, positive and negative predictive values with 95%CIs were calculated for Xpert MTB/RIF against culture and composite reference standard (made of Xpert, culture and clinical improvement after anti-TB treatment) using MedCalc software. Chi-square test was used to check the association between test statuses. P-values less than 0.05 were considered as statistically significant.

4.11. Ethical Considerations

Ethical clearance (Protocol number IHRPGD552/18) was obtained from Institutional Review Board of Jimma University Institute of Health. Letter of permission to conduct the study was obtained from JMC clinical director office. Written informed consent was obtained from the parents/care takers of the children. Concerning the confidentiality of the result, since all clients who participated in the study had unique code number, confidentiality was kept well throughout the study. The results of laboratory findings were communicated to the treating physicians for patient managements.

4.12. Dissemination Plan

The finding of the study will be disseminated to JU Department of Medical Laboratory Sciences, Department of Pediatrics and Child Health, JMC, Federal Ministry of Health, Oromia Regional Health Bureau, zonal and district health offices, and district administration of the study area. The finding will also be submitted to peer reviewed journals for publication so as to serve as baseline for further studies.

5. RESULTS

5.1. Socio-Demographic Characteristics of the Participants

Majority of the study participants, 51.3% (78/152) were female and 48.7% (74/152) male. Most of the study participants were in the age category of 1-4years accounting for 52% (79/152). Majority, 64.5% (98/152) and 35.5% (54/152) of the participants were from rural and urban residents, respectively see (**Table 2**).

A total of 178 children enrolled to the study. These comprise 17 expectorated sputum, 161 GA and 152 stool specimens. All expectorated sputum and GA specimens were tested by Xpert MTB/RIF assay and LJ culture. Twenty six patients with an Xpert assay final results of negative were excluded from the final analysis i.e. (17 Xpert and culture negative) patients who did not provide stool specimen and (9 Xpert negative and culture contaminated cases). The remaining 152 respiratory specimens which were 17sputum and 135 GA included in the final analysis. Stool specimens were tested only by Xpert assay. Of the total 152 stool specimen run by Xpert assay, 4.6% (7/152) of the specimen with an Xpert final result of 4 error and 3 invalid and they were excluded from further analysis.

The overall, microbiologically and clinically confirmed childhood TB case in this study was 11.8% (20/169). TB was microbiologically confirmed in 5.9% (10/169). Of these, 9 LJ culture and 8 Xpert positive TB case, respectively. Moreover, TB was clinically confirmed in 6.3% (10/159) of microbiologically negative cases.

Table 2: Demographic Characteristics of Participants with their Diagnostic Results

Variable	Culture positive n(%)	Culture negative n(%)	GA Xpert positive n(%)	GA Xpert negative n(%)	Stool Xpert positive n(%)	Stool Xpert negative n(%)	P-value
Age (years)							
< 1	1(0.7)	13(7.2)	1(0.7)	11(7.2)	1(0.7)	11(7.2)	0.7
1-4	6(3.9)	73(48)	5(3.3)	74(48.7)	7(4.6)	68(44.7)	
5-10	1(0.7)	38(25)	1(0.7)	38(25)	1(0.7)	36(23.7)	
11-14	1(0.7)	21(13.8)	1(0.7)	21(13.8)	1(0.7)	20(13.2)	
Gender							
Male	0	74(48.7)	1(0.7)	73(48)	1(0.7)	68(44.7)	0.03
Female	9(5.9)	69(45.4)	7(4.6)	71(46.7)	9(5.9)	67(44.1)	
Residence							
Urban	2(1.3)	52(34.2)	1(0.7)	53(34.9)	2(1.3)	49(32.2)	0.4
Rural	7(4.6)	91(59.9)	7(4.6)	91(59.9)	8(5.30	86(56.6)	

5.2. Clinical Characteristics and other related Information of the Participants

Of the 152 children screened for PTB, 92.8% (141/152) had complaint of cough >2 weeks, 80.3% (122/152) had loss of appetite, 71.7% (109/152) had fever, 59.2% (90/152) had weight loss, 55.9% (90/152) had weakness/fatigue, 46.1%(70/152) had shortness of breath, 23% (35/152) had TB contact history and 12.5% (19/152) had chest pain see **(Figure 3**).

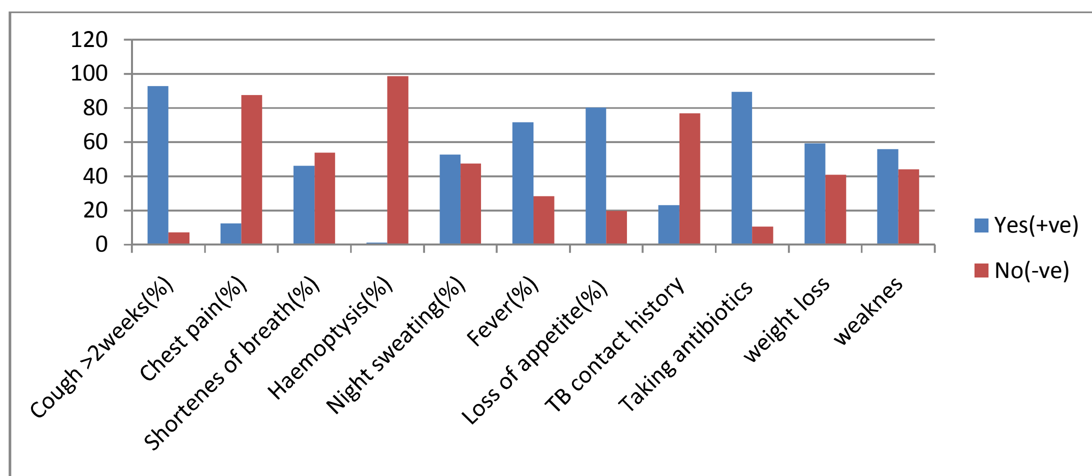

Figure 2: Clinical Features of the Study Participants

Clinical features used for the diagnosis of PTB (in percentage). In this figure Yes (+Ve) is positive to clinical features and No (−Ve) implies the absence of clinical features.

Majority of the participants were vaccinated for BCG 93.4% (142/152) and 6.6% (10/152) unvaccinated. Participants, 35.5% (54/152) were severely malnourished, 11.8% (18/1520 moderately malnourished and 52.6% (80/152) well nourished. Six (3.9%) of the 152 children had known HIV positive test result and they were on Anti-retroviral therapy (ART) with stage one see **(Table 3)**.

Table 3: Other related Clinical Information's of the Participants

Characteristics	Culture positive n(%)	Culture negative n(%)	GA Xpert positive n(%)	GA Xpert negative n(%)	Stool Xpert positive n(%)	Stool Xpert negative n(%)	P-value
CXR findings							
Consistent TB	5(3.3)	4(2.6)	6(3.9)	3(2)	6(3.9)	3(2)	0.00
Not consistent	4(2.6)	86(56.6)	2(1.3)	88(57.9)	4(2.6)	81(53.3)	
Normal	0	53(34.9)	0		0	51(33.6)	
BCG status							
Vaccinated	9(5.9)	133(87.5)	8(65.3)	134(88.2)	10(6.6)	125(82.2)	0.4
unvaccinated	0	10(6.6)	0	10(6.6)	0	10(6.6)	
Nutritional status							
SAM	6(3.9)	48(31.6)	6(3.9)	48(31.6)	7(4.6)	43(28.3)	0.2
MAM	1(0.7)	17(11.2)	1(0.7)	17(11.2)	1(0.7)	17(11.2)	
Normal	2(1.3)	78(51.3)	1(0.7)	79(52)	2(1.3)	75(49.3)	
HIV status							
Positive	0	6(3.9)	0	6(3.9)	0	6(3.9)	0.53
Non-reactive	9(5.9)	137(90.1)	8(5.3)	138(90.8)	10(6.6)	129(84.9)	

CXR= chest X-ray, BCG= Bacille Calmette-Guérin, SAM= severe acute malnutrition, MAM= moderate acute malnutrition. All the above additional clinical data of the study participants were gathered from their documented medical records. CXR findings were interpreted by the radiologists and documented on the patient's medical records. SAM documented by the clinicians as midupper arm circumference (MUAC) < 115 mm. MAM is MUAC between 115mm and <125mm. Normal; MUAC over 135mm the child is well nourished.

5.3. Detection Rate of Pulmonary Tuberculosis

Of the 152 valid Xpert assay respiratory results, 5.3% (8/152) of the patients were positive for MTBC. Of the 8 Xpert assay positive results, seven of the eight were also positive by LJ culture. GA Xpert testing gave positive result for one (0.7%) specimen which was negative

by LJ culture. Additionally, GA Xpert gave negative result for two (1.3%) specimens who were positive by LJ culture see (**Table 4**). There were no cases of rifampin resistance was detected by Xpert MTB/RIF in both respiratory and stool specimen testing.

Valid Xpert MTB/RIF assay results were reported for 145 stool specimens, out of which 6.9% (10/145) of the specimens were positive for MTBC. Of the 10 Xpert positive stool specimens, nine of the ten specimens were also positive by GA culture. Stool Xpert gave positive result for one (0.7%) specimen which was negative by GA culture see (**Table 4**).

Table 4: Proportion of GA Xpert, Stool Xpert and GA Culture

	GA Culture			**p-value**
GA Xpert	Positive n(%)	Negative n(%)	Total n(%)	
Positive	7(4.6)	1(0.7)	8(5.3)	0.00
Negative	2(1.3)	142(93.4)	144(94.7)	
Total	9(5.9)	143(94.1)	152(100)	
		GA Culture		
Stool Xpert	Positive n(%)	Negative n(%)	Total n(%)	
Positive	9(6.2)	1(0.7)	10(6.9)	0.00
Negative	0	135(93.1)	135(93.1)	
Total	9(6.2)	136(93.8)	145(100)	

Of the 8 Xpert MTB/RIF positive GA specimen, stool Xpert detected 100% (8/8) without missing any positive from GA Xpert testing. Stool Xpert gave positive result for two (1.4%) specimens which were found to be negative by GA Xpert testing see **(Table 5)**.

Table 5: Proportion of Stool and GA Xpert MTB/RIF

	GA Xpert			**P-value**
Stool Xpert	Positive n(%)	Negative n(%)	Total n(%)	
Positive	8(5.5)	2(1.4)	10(6.9)	0.00
Negative	0	135(93.1)	135(93.1)	
Total	8(5.5)	137(94.5)	145(100)	

5.4. Diagnostic Classifications of the Participants

Out of 142 patients, found to be negative with microbiological tests, 7% (12/142) of them were clinically diagnosed as TB by the clinicians. Of the 12 clinically diagnosed TB cases, 83.3% (10/12) of them were clinically improved after ATT which was documented on their medical records by the clinicians and considered as TB, whereas the remaining two of the twelve clinically diagnosed TB cases were not clinically improved and considered as non TB cases.

Out of the 10 clinically improved cases, four of the ten cases were radiological findings suggestive of TB which were interpreted by the radiologists and documented on the patient's medical records and considered as "probable TB". Six of the ten clinically improved cases were no radiological evidences of TB documented on their medical records and considered as "possible TB". The remaining 91.5% (130/142) of the cases were ruled out for TB disease and alternative diagnoses were available and considered as "non TB" see **(Table 6)**.

Table 6: Proportion of Clinically Diagnosed and Microbiologically Confirmed TB Cases

	CRS				
Stool-Xpert	**Confirmed-TB** n(%)	**Probable-TB** n(%)	**Possible-TB** n(%)	**not-TB** n(%)	**Total** n(%)
Positive	10(6.9)	0	0	0	10(6.9)
Negative	0	4(2.8)	6(4.1)	125(86.2)	135(93.1)
Total	10(6.9)	4(2.8)	6(4.1)	125(86.2)	145(100)
GA-Xpert					
Positive	8(5.3)	0	0	0	8(5.3)
Negative	2(1.3)	4(2.6)	6(3.9)	132(86.8)	144(94.7)
Total	10(6.6)	4(2.6)	6(3.9)	132(86.8)	152(100)
GA-Culture					
Positive	9(5.9)	0	0	0	9(5.9)
Negative	1(0.7)	4(2.6)	6(3.9)	132(86.8)	143(94.1)
Total	10(6.6)	4(2.6)	6(3.9)	132(86.8)	152(100)

5.5. Performance of Xpert MTB/RIF assay Compared to LJ Culture

Using GA culture as a reference standard, the sensitivity, specificity, PPV and NPV of GA Xpert MTB/RIF had 77.8% (95%CI: 40-97.2), 99.3% (95%CI: 96.2-100), 87.5% (95%CI: 49-98.1) and 98.6% (95%CI; 95.5-99.6), respectively. Whereas, the sensitivity, specificity,

PPV and NPV of stool Xpert MTB/RIF assay was 100% (95%CI: 66.4-100), 99.3% (95%CI: 96.2-100), 90% (95%CI: 56.1-98.5) and 100%, respectively see **(Table 7)**.

Table 7: Performance of Xpert MTB/RIF Compared to Culture

Tests	Culture as a reference standard			
	Sensitivity % (95%CI)	**Specificity % (95%CI)**	**PPV % (95%CI)**	**NPV % (95%CI)**
GA Xpert	77.8(40-97.2)	99.3(96.2-100)	87.5(49-98.1)	98.6(95.5-99.6)
Stool Xpert	100(66.4-100)	99.3(96.2-100)	90(56.1-98.5)	100

GA= gastric aspirate, CI= confidence interval, PPV= positive predictive value, NPV= negative predictive value

5.6. Performance of Xpert MTB/RIF Compared to CRS

Using CRS as a reference standard, the sensitivity, specificity, PPV and NPV of stool Xpert was 50% (95%CI; 27.2-72.8), 100% (95%CI; 97.1-100), 100% and 92.6% (95%CI; 89-95.1), respectively. Whereas, the sensitivity, specificity, PPV and NPV of GA Xpert was 40% (95%CI: 19.1-64), 100% (95%CI; 97.2-100), 100% and 91.7% (95%CI: 88.5-94), respectively see **(Table 8)**.

Table 8: Performance of Xpert MTB/RIF Compared to CRS

Tests	CRS			
	Sensitivity % (95%CI)	**Specificity % (95%CI)**	**PPV % (95%CI)**	**NPV % (95%CI)**
GA Xpert	40(19.1-64)	100(97.2-100)	100	91.7(88.5-94)
Stool Xpert	50(27.2-72.8)	100(97.1-100)	100	92.6(89-95.1)
GA culture	45(23.1-68.5)	100(97.2-100)	100	92.3(89-94.7)

GA= gastric aspirate, CI= confidence interval, PPV= positive predictive value, NPV= negative predictive value, CRS= composite reference standard

6. DISCUSSIONS

The current study finding showed that overall 11.8% (20/169) of microbiologically and clinically confirmed TB cases. This is lower than other study conducted in Pakistan reported 52% (26/50) of the overall microbiological and clinically diagnosed TB cases (55). This possibly, due to the different screening tool (modified KJ score) used by the study to recruit children with high likelihood of TB.

In the present study, the sensitivity of stool Xpert MTB/RIF was 100% compared to culture confirmed GA specimens. This is comparable with other studies conducted in Ethiopia, Kenya and Pakistan which have reported sensitivities ranging from 88.9 to 100%, (51),(56),(55) respectively. However, the sensitivity of stool Xpert MTB/RIF in the current study is higher than the study conducted in South Africa which have reported sensitivity of 45.5% (20). This may be due to different stool Xpert testing protocol method we have used. While, the sensitivity of GA Xpert was 77.8% compared to culture. This is comparable with a study conducted in Zambia which have reported the sensitivities of 68.8% (57). There were 2 stool Xpert positive specimen which were negative in GA Xpert testing. This may be due to very low bacilli load in GA specimen. Stool Xpert testing was 100% positive without missing any positive cases in GA Xpert and culture tests. The finding suggests that stool is an alternative specimen for diagnosis of PTB in children who unable to produce sputum specimen.

The pooled sensitivity of stool Xpert MTB/RIF was decreased to 50% when compared to CRS. This is comparable with other studies conducted in South Africa and Pakistan which have reported sensitivities ranging from 47.1 to 58.8% (43),(55). The decreasing of sensitivity in Xpert MTB/RIF assay when compared to CRS is due to both culture and Xpert MTB/RIF tests can detect cases of high bacterial loads which missed positive cases in children due to the presence of low bacterial loads in the specimen of children. In the current study, most of the clinically diagnosed TB cases were improved with empirical ATT without microbiological confirmation, thus Xpert and culture negative cases were not excluded TB in children.

The specificity of stool Xpert had 99.3% when compared to culture which is consistent with other previous studies conducted in Ethiopia, South Africa and Egypt which have demonstrated specificities ranging from 99.3 to 99.7% (20), (51), (58). However, the pooled specificity of stool Xpert MTB/RIF was 100% when compared to CRS which is similar with

other study conducted in Pakistan (55). One culture negative specimen was positive in stool and GA Xpert MTB/RIF testing. This may be due to the presence of non-viable bacilli due to the harsh decontamination process of GA specimens.

In the current study, stool Xpert MTB/RIF testing was performed by simple stool testing protocol adapted from KNCV TB foundations (54), which was omitted some labor intensive procedures such as homogenization, decontamination and centrifugation done by other studies (56), (55), (58). Additionally, the simple stool processing methods we used can be reduced time of sample processing, minimized the workload of laboratory personnel and costs. Stool specimen is easy to obtain from young children without any invasive procedures, if stool replaces the GA—Xpert testing can be implemented in peripheral health facilities, enable to allow rapid initiation of ATT and thereby reducing mortality and TB transmissions of children.

In the present study, the sensitivity of Xpert MTB/RIF significantly reduced when compared against the CRS, with only around 50% of clinically diagnosed TB cases being detected in stool spacemen. Hence, a negative Xpert MTB/RIF does not always exclude a diagnosis of PTB in children. Patients whose symptoms and signs strongly suggest PTB should be started ATT, despite a negative Xpert MTB/RIF. Thus, clinical diagnosis remains an essential part of the diagnostic pathway until better and more sensitive tools are available.

Our study has some limitations one of which is the small sample size due to time and resource constraints. Additional limitations of our study are the fact that, we used LJ medium for mycobacteria culture rather than the more sensitive liquid MIGIT medium due to shortage of the medium, the fact that we collected only single respiratory and stool specimens rather than successive specimens which could have probably increased the yields of the test done.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

Stool Xpert assay has higher sensitivity (100%) and specificity (100%) compared to LJ culture and CRS, respectively. However, Xpert assay could not able to detect TB in clinically diagnosed children, so it should not be used a standalone test for diagnosis of TB in children. Children with a high clinical probability of TB despite a negative Xpert should be started on anti-TB treatment until better and more sensitive tools are available.

7.2. Recommendations

Introduction of stool Xpert MTB/RIF testing as the routine TB diagnosis for children with presumptive PTB might be a solution to the current challenges of getting respiratory specimens from children, particularly for high-burden low resource settings like Ethiopia.

Further study with a larger sample size with additional specimen overcoming the limitations of our study is required. We also recommend further studies that assess the impact of the use of Xpert MTB/RIF on time to TB diagnosis and clinical outcomes, thereby permitting cost–effectiveness analyses to be performed.

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9. ANNEX

Annex 1. Informed Consent (English version)

Part I Information sheet

Hello? My name is **Mitiku Dubale.** I am currently a student of Jimma University, Institute of Health, School of Medical Laboratory Sciences, and Department of Medical Microbiology. I am here to undertake study about Xpert MTB/RIF assay for the diagnosis of childhood pulmonary tuberculosis using stool specimen.

The main objective of this study is to assess the "diagnostic performance of Xpert MTB/RIF assay on stool sample from children with clinically suspected of having pulmonary tuberculosis". And this questionnaire is intended to gather physical and clinical features of pulmonary tuberculosis. If you agree, clinical sample (gastric aspirate) will be collected from your child by experienced hospital staff member in the early morning hour, as part of routine diagnosis and stool sample will be collected at the time of child defecate. During gastric aspiration, your child may feel some discomfort but this will not produce any serious problems. These procedures are routinely practiced for TB diagnosis and the collected specimen will be used for diagnostic as well as research purpose. All information you provide will be handled as confidential and your individual answers will not be known, except by the interviewer and the coordinator of this study.

As a participant in this study, you (your child) will not pay money for specimen collection and laboratory expenses. The results of laboratory examination will be reported back to the treating physician for your child management. Participation in the study is on voluntary basis and you/your child have the right to refuse to participate in the study and withdraw your consent/assent at any time without any prejudice to your child case. It will in no way affect your/your child's right to acquire health care and treatment if you do not allow your child wish to participate in this research.

If you allow your child to participate in this study, you need to understand and sign the consent form. If you have questions, you have the right to get proper explanation. We thank you for your participation in the study.

Are you willing to participate in the study? 1. Yes 2. No

Contact information

Principal investigator: **-** Mitiku Dubale (MSc candidate)

Address: - Jimma University Institute of Health Sciences, School of Medical Laboratory sciences. Mobile phone: +251905996131/email: mitikufast@gmail.com

Part II Parent or guardian informed consent

I have been invited to have my child participate in the research of childhood pulmonary tuberculosis diagnosis. I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for my child to participate as a participant in this study.

____________________________	____________________	______________
Name of Parent/Guardian	Signature	Date
____________________________	____________________	______________
Name of data collector	Signature	Date

Part III Assent Form for children aged 10 - 14 years

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I would participate in this study provided my parents/guardians give their consent**.** To give my body fluid (Gastric aspirate / sputum) and stool samples to inform my permission on providing the specimens and be a participant in this study and understand that I have the right to withdraw from the study at any time.

Name of participant--

Signature---

Date ---

Annex 2. Informed consent form (Amharic version)

<u>የፈቃደኝነት መጠየቂያ ቅጽ</u>

የጥናት ርዕስ፡- በሳንባ ነቀርሳ በሽታ ከተጠረጠሩ ህጻናት የሰገራ ናሙና በመጠቀም በXpert MTB/RIF ማመርማርያ መሳሪያ የሳንባ ነቀርሳ በሽታን ምርማራ ማድረግ

ክፍል I የመረጃ ዝርዝር

ስሜ **<u>ምትኩ ዱባለ ይባላል</u>፡፡** በአሁኑ ሰዓት በጅማ ዩኒቨርሲቲ በጤና ኢንስቲትዩት በሕክምና ላቦራቶሪ ሳይንስ ትምህርት ቤት በሕክምና ስነ-ረቂቅ ተህዋስያ ትምህርት ክፍል ሁለተኛ ዓማት የሁለተኛ ዲግሪ ተማሪ ነኝ።

የዚህ ጥናት ዋና ዓላማ በተለምዶ የአክታ ወይም የሰዉነት ፈሳሽ ናሙና በመጠቀም የሳንባ ነቀርሳ በሽታን ከህጻናት ምርማራ ስደረግ ቆይተል፡፡ ነገር ግን እነዚህን ናሙና ከ ህጻናት ማግኘት አስቸጋር ስለሆኔ በዚህ ጥናት የሰገራ ናሙና በመጠቀም የህጻነት ሳንባ ነቀርሳ በሽታን በXpert MTB/RIF መሳሪያ ማመርመር ያስችል እንደሆነ እና ማሳሪያዉ ምን የህል በትክክል በሽተዉን ማመርመር እንደምችል ለማረጋገጥ ነዉ፡፡

ለዚህ ምርመራ የመጣችሁ ታካሚዎች ስለ በሽታዉ በተመለከተ የተዘጋጀ ትንሽ የአፍ መጠይቅ ስላለ የእረስዎ ትክክለኛ ምላሽ ለምርመራዉ ስለምያስፈልገን የእርስዎን ትብብር በአክብሮት አንጠይቃለን፡፡ በመቀጠል ለምርመራ ከምሰጠዉ መደበኛ የሰዉነት ፋሳሽ ወይም አክታ ናሙና በተጨማሪ ለዚህ ጥናት የሚያስፈልገዉን የሰገራ ናሙና ከተክሚዉ የምትሰጡ መሆኑን ልንገልጽላችሁ እንወዳለን፡፡ የሚትሰጡት የግል መረጃዎች በሙሉ ምስጢራዊነቱ የሚጠበቅ ይሆናል፡፡

በዚህ ጥናት ተሳታፊ ሲሆኑ ለልጅዎ ለሚደረግለት ምርመራ ገንዘብ አይከፍሉም፡፡ በጥናቱ ውስጥ የሚደረግ ተሳትፎ በፈቃደኝነት ላይ የተመሰረተ ሲሆን ልጅዎ በጥናቱ ዉስጥ እንድቀጥል ካልፈለጉ ስምምነተዎን ያለምንም ጥያቄ የማቀረጥ ሙሉ መብት አለዎት፡፡ ልጅዎ በዚህ ጥናት እንዲሳተፍ ካልፈቀዱ የልጅዎ የጤና እንክብካቤ እና ህክምና የማግኘት መብት ላይ ምንም ተጽዕኖ አይኖረውም ፡፡ ልጅዎ በዚህ ጥናት ውስጥ እንዲሳተፍ የሚፈቅዱ ከሆነ በስምምነት ቅጽ ላይ መፈረም አለብዎት፡፡ ጥያቄ ካሉዎት የመጠየቅ እና ትክክለኛ ማብራሪያ የማግኘት መብትዎ የተጠበቀ ነዉ፡፡

በጥናቱ ለመሳተፍ ፋቃደኛ ነዎት? 1. አዎ 2. አይደለም

አድራሻ

የጥናቱ ዋና ተመራ ማሪ: - ምትኩ ዱባለ

አድራሻ - ጂማ ዩኒቨርሲቲ የጤና ሳይንስ ተቋም የህክምና ላቦራቶሪ ሳይንስ ትምህርት ቤት

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ክፍል ሁለት፡- የወላጅ ወይም አሳዳጊ የስምምነት ቅጽ

ከላይ የተጠቀሱትን መረጃዎች አንብቤ ወይም ተነቦልኝ ስለመረጃዉ ጥያቄ የመጠየቅ እና ለጥያቀዉ በቂ ምላሽና ማብራሪያ እነደምስጠኝ እድል አግኝቻለሁ፡፡ ስለዚህ ልጄ በዚህ ጥናት ተሳታፊ እንዲሆን በፋቃደኝነት መስማማቴን በፊርማዬ አረጋግጣለሁ፡፡

______________________________	__________________	________________
የወላጅ / አሳዳጊ ስም	ፊርማ	ቀን
__________________________	___________________	________________
የመረጃ ሰብሳቢ ስም	ፊርማ	ቀን

ክፍል ሦስት ፡- ዕድሜያቸው ከ 10 - 14 ዓመት ለሆኑ ህጻናት የተዘጋጀ የስምምነት ቅጽ

በዚህ ጥናት ውስጥ እንዲሳተፍ ወላጆቼ ወይም አሳዳጊዎቼ በፈቃደኝነት የእነሱን ስምምነት እነደሰጡ እኔም በፈቃደኝነት የጥናቱ ተሳታፊ ለመሆን ማስማማቴን እየገለጽኩ ለጥናቱ የሚያስፈልገዉን የምርመራ ናሙና በተጠየኩ ጊዜ ና ሰዓት ለመስጠት ማስማማቴን በፊርማዬ አረጋግጣለሁ፡፡

የታካምዉ ስም ---------------------------------------

ፊርማ --

ቀን --

Annex 3. Informed Consent (Afan Oromo version)

Guca Gaaffii Fedhii Hirmaannaa

Gucni kun kan maatii ijoollee waggaa shanii gadii ta'an Giddugala meedikaalaa Yuunivarsiitii Jimmaattii yaalsisaa jiraniif qophaa'edha

Gucni Gaaffii Fedhii Hirmaannaa kun Kutaalee lama qaba: Isaanis

- Kutaa waa'ee qorannichaa gadi-fageenyaan ibsu fi
- Kutaa ragaa fedhii hirmaannaa mallattoodhaan ibsu

Kutaa 1ffaa:- Waa'ee qorannichaa

Seensa

Ani ragaa funaanaa qorannoo mata duree armaan olitti geggeeffamu kanaa yoon ta'u, qorannoo Dhukkuba TB daa'immanii/Dhibee sombaa/ qabaniif meeshaa GeneXpert jedhamuun taasisuuf deemna.Kanaafuu mucaan keessan qorannoo kana irratti akka hirmaatuuf fedhii keessan ibsuuf waa'ee qorannoo kanaa gadi fageenyaanan isiniif ibsuu barbaada.Murtoo hirmaanaa mucaa keessanii amma kana murteessuun dirqama miti, yeroo itti amantanitti murtessuu dandeessu.Nama isin barbaaddan waliinis waa'ee qorannoo kanaa mar'achuu dandeessu.Gaaffiilee isin hubachuu dhabdaniifis an isiniifin ibsa.

Akkataa Filannoo Hirmaattota Qorannoo kanaa

Mucaan keessan qorannoo kana irratti akka hirmaatuuf kan filatame, yeroo kana dhukkutaTB qabaachuu waan danda'uufii jiraataa naannoo kanaa fi maatiin keessan tajaajilamaa giddugala medikaaalaa yuunivarsiitii Jimmaa waan taataniif qofadha. Haaluma kanaan ijoolleen waggaa shanii gadii dhibee kanaan shakkaman hundi, yeroo qorannoo kana keessatti carraa qorannoo kana irratti hirmaachuu ni qabu.

Duraa duuba qorannichaa

Mucaan keessan qorannoo kana irratti akka hirmaatuuf yoo eyyamtan, gaaffillee afaanii tokko tokko kan isin gaafannuu fi qorannoon laaboraatorii mucaa keessanii akka taasifamu kan nu gargaartan ta'a.

Dhiibbaa Qorannoo Keessatti Hirmaachuun Ykn Hirmaachuu Dhabuun Qabu

Sababa mucaan keessan qorannoo kana irratti hirmaateef/tteef rakkoon isinirra gahu tokkoyyuu hin jiru.

Faayidaa Qorannoo Keessatti Hirmaachuun Qabu

Qorannoo kana keessatti hirmaachuu keessaniif faayidaan kallattiin isin argattan hin jiru.Haa ta'u malee bu'aan qorannoo kana irraa argamu isiniifis ta'ee hawaasa naannoo keessaniitiif dhukkuba TB daa'imman waggaa shanii hir'isuuf gahee guddaa taphata.

Iccitii Ragaalee

Ragaaleen qorannoon kanaaf jecha isin nuuf laattanis ta'ee kan nuti qoranne arganne hundi iccitiidhaan ni taa'u. Waraqaa gaaffii kana irratti maqaan keessanis ta'ee kan mucaa keessanii kan hin caqasamnee fi koodii namni biraa beekuu hin dandeenye kan qabuudha.

Mirga Hirmaachuu Dhabuu

Yoo hirmaannaan mucaa keessaniitti hin amanne ta'e, fedhii hirmaannaa agarsiisuun dirqama miti. Qorannoo kana irrattti hirmaachuuf fedhii dhabuu kessaniin kan walqabee tajaajila yaala gahaa argachuu keessan irratti dhiibbaa tokkollee kan hin qabne ta'uu hubadhaa. Isiniif mucaan keessan tajaajila barbaachisu hunda argachuuf mirga guutuu qabdu.

Namni Quunnamuu barbaaddan yoo jiraate

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Kutaa 2ffaa: Waraqaa Ragaa Fedhii Hirmaannaa

Akkaataa odeeffannoo armaan olitti naaf dubbifame kanaan qorannoo mata duree "Qorannoo Dhukkuba TB daa'immanii/Dhibee sombaa/ meeshaa GeneXpert jedhamuun" jedhu irratti aniif mucaan koo akka hirmaannuf fedhiin qabu mallattoo kootin nan mirkanneessa.

Maqaa Maatii Mucaa_______________________________

Mallattoo__________________

Guyyaa___________________

Annex .4. Data collection tool

Date _____________

S/No	Questions	Response category
1	Identification of the patient	1. Patient card No_____________ 2. Lab Code: _________________
2	Age	
3	Gender	1. Male 2. Female
4	Place of residence	1. Urban 2. Rural
5	BCG status	1. Vaccinated 2. Not vaccinated
6	Cough	1. Yes 2. No
7	If yes to Q6, duration of cough	---------------------Days /Weeks/ Month
8	Chest pain	1. Yes 2. No 3. NA
9	Shortness of breath	1. Yes 2. No
10	Haemoptysis	1. Yes 2. No
11	Night sweating	1. Yes 2. No
12	Loss of appetite	1. Yes 2. No
13	Fever	1. Yes 2. No
14	Weakness/fatigue/less active	1. Yes 2. No
15	Weight loss	1. Yes 2. No
16	Nutritional status	1. Severe malnutrition

		2. Moderate malnutrition 3. Mild malnutrition 4. Normal
17	Taking antibiotic treatment in the last 2weeks	1. Yes 2. No 3. Unknown
18	TB contact history in the past 2 years	1. Yes 2. No 3. Unknown
19	Current anti-TB treatment	1. Yes 2. No 3. Unknown
20	Past History of anti-TB treatment	1. Yes 2. No 3. Unknown
21	If yes, to Q22 , had he/she complete treatment	1. Yes 2. No 3. Unknown
22	Chest X- ray finding	1. Hilar/mediastinal lymphadenopathy 2. Miliary infiltrate 3. Cavitation 4. Pleural or pericardial effusion 5. Not consistent with TB 6. Normal
23	HIV sero-status	1. Reactive 2.Non-Reactive 3. Unknown
	Laboratory findings	
24	GeneXpert MTB/RIF (GA/sputum) result	1. MTB Detected 2. MTB Not Detected
25	If MTB detected, to Q27 RIF-R result	1. RIF-R Detected 2. RIF-R Not Detected
26	GeneXpert MTB/RIF (stool) result	1. MTB Detected 2. MTB Not Detected
27	If MTB detected, to Q29 RIF-R result	1. RIF-R Detected 2. RIF-R Not Detected

28	Culture (L-J solid media) result	1. Positive 2. Negative 3. Contaminated 4. NTM
29	TB diagnostic classification	1. Confirmed TB 2. Probable TB 3. Possible TB 4. Not-TB

Name of data collector---------------------------------- Sign---------------------------------- Date--------------------

Name of investigator --------------------------------------Sign ------------------------------- Date -------------------

DECLARATION

I the undersigned, declare that this is my original work and has never been presented for the degree in this or any other university and all the source materials used for this thesis have duly acknowledged.

Name: Mitiku Dubale

Signature: ___________

Name of the institution: Jimma University Institute of Health Sciences

Date of submission: _______________________

This thesis has been submitted for examination with my approval as University advisor.

Name and signature of the first advisor

Name: Mulualem Tadesse (PhD, Assistant Prof. of Clinical Microbiology)

Signature ________________________

Name and signature of the second advisor

Name: Melkamu Berhane (MD, Associate Prof. of Pediatrics and Child Health)

Signature ________________________