ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PREVALENCE OF EXTRAPULMONARY TUBERCULOSIS AND RIFAMPICIN RESISTANT IN CHILDREN AT SELECTED HEALTH FACILITIES OF SOUTH WEST SHEWA ZONE, OROMIA, ETHIOPIA

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A THESIS SUBMITTED TO THEDEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF ALIED HEALTH SCIENCES, COLLEGE OF HEALTH SCIENCE, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF MASTER OF SCIENCE DEGREE IN CLINICAL LABORATORY SCIENCES (DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY)

JUNE, 2018

ADDIS ABABA, EHIOPIA

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

This is to clarify that the thesis is prepared by Azmach Biset, which is entitled Prevalence of *Extra pulmonary tuberculosis and rifampicin resistance in children at selected Health facilities of South west shewa zone ,Oromia Ethiopia*, and submitted in partial fulfillment of the requirements for the degree of Masters of Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Approved by the Examining Board

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Chairman, Dep. Graduate Committee Signature Date

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External Examiner Signature Date

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Internal Examiner Signature Date

Acknowledgment

Foremost, I would like to express my sincere gratitude to my advisors **Mr. Kassu Desta** for the continuous support, his patience, motivation, and constructive criticism. His guidance helped me in all stage of the study. I would like also to acknowledge and thank **AdisAbeba University College of Health Sciences, School of Applied Health Sciences, and Department of Medical Laboratory Sciences** for giving me such a chance to assess the TB infection in such communities of study area

I would like also to acknowledge my co-advisors **Mr.Mohamed Suadi** who provide me editorial work of the manuscript. This study would not have been completed without the support of Ethiopian Public Health Institute (EPHI).

I am indebted to thank **Mr.Ephrem Tesfaye** (EPHI TB Lab Head) for allowing me to use EPHI TB lab resource and covering all related cost. My gratitude also goes to TB lab staffs of EPHI (**Hilina Molalign and Shewki Moga**) for processing the TB culture.

Special thanks goes to **St.luke Catholic Hospital, TullubolоHospital, AmeyaHospital, Atat Hospital, Woliso 1&2 HC, Dilela HC, lab staffs besides The Pediatricians and neonatologists, Internists, and the study participants** for their contribution in specimen and data collection.

I would like also to thank and acknowledge the **study participants** without whom the study cannot be accomplished by providing their willingness to participate in this study

I would like to thank St.luke catholic Hospital laboratory staffs and CUAMM (Italian NGO **Dr Gaetano Azzimonti,(medical director) Sr.Clara Rosalin (CEO).**

Finally I would like to thank my families, parents and those who were with me until the end of my education.

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List of Abbreviations

ART	Antiretroviral treatment
BAL	.Bronchoalveolar lavage (bronchial washing)
BCG	Bacillus Calmette Guerin
CFU	Colony Forming Units
Ct	Cycle threshold
CDC	Center for Disease Control
CSF	Cerebrospinal fluid
DST	Drug susceptibility testing
EPTB	Extra pulmonary tuberculosis
GLA	Gastric lavage
HBCs	.High-burden countries
HIV	Human immune deficiency virus
IS	Induced sputum
MDR-TB	Multi drug resistance TB
MGIT .	Mycobacterium growth indicator tube
MTB	Mycobacterium tuberculosis
MTBC	Mycobacterium tuberculosis complex
PCR .	Polymerase chain reaction
PI .	Principal investigator
RIF	.Rifampecin resistance
SR	Sample treatment reagent
SOPs	Standard Operational Procedures
SPSS	Statistical Package for Social Sciences
TB	Tuberculosis
WHO	World health organization

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Abstract

Background: Tuberculosis (TB) is a chronic infectious disease that has long been one of the major health problems. It affects individuals of all ages and both sexes. Poverty, malnutrition and over-crowded living conditions have been known for decades to increase the risk of developing the disease .Extra pulmonary tuberculosis (EPTB) is an important clinical problem defined as the isolated occurrence of tuberculosis in any part of the body other than lungs. Aim of the study is to determine the Prevalence of EPTB Cases in children.

Objectives: Prevalence of Extra pulmonary Tuberculosis and Rifampicin Resistant in children at selected health facilities of South West Shewa Zone, Oromia, Ethiopia

Materials and Methods: A crossectional study was conducted from December 20, 2017-April 26 2018. A total of 416 samples (Pleural, Gastric lavage, CSF, Pus, Peritoneal fluid, Lymph node aspirate, Synovial fluid, and Ascitic fluids) from patients with signs and symptoms of suggestive of tuberculosis at St.luke,Tullubolo,Ameya,Atat hospitals and Woliso, Dilela Health centers from December 20,20171, 2018 to April 26 25,2018 were recruited by consecutive sampling method. Extra pulmonary Tuberculosis samples were tested directly by Xpert MTB/RIF assay and MGIT and those extra pulmonary suspected samples were cultured for isolation and drug sensitivity testing of TB bacilli. Results with contaminated cultures (for both LJ and MGIT) were excluded from analysis. Data entry and analysis was done using SPSS statistical software version 20.

Result: Out of 416 body fluid specimens, 15.8% (66/416) were positive for MTB using Xpert MTB/RIF assay and no specimen were exhibited as a resistance to RIF. 10.57% 44/397) cases of Xpert MTB/RIF assay positive were MGIT positive, 3.12% (13/416) cases of Xpert MTB /RIF assay positive were MGIT negative and 2.2% (9/416) cases of Xpert MTB/RIF assay negative were MGIT positive .The most crucial risk factor that was significantly associated with extra pulmonary tuberculosis infection in this study was HIV.

Conclusion: There was a significant difference between GeneXpert results compared with Culture results. A significant proportion of EPTB cases were also co-infected with HIV.A more accurate test could contribute EPTB case detection, and thus reducing the morbidity and mortality

Keyword: Liquid culture, extra pulmonary tuberculosis, rifampicin resistance

1 INTRODUCTION

1.1 Background:-

Tuberculosis (TB) is a chronic infectious disease mainly caused by *Mycobacterium tuberculosis.* Mycobacterium tuberculosis (MTB), the causative agent of the disease, is of great global epidemic importance. The bacterium affects not only lungs, but also the other parts of the body system which is generally termed as extra pulmonary tuberculosis [1]. Children account for 6 %– 10 % of all TB cases worldwide. TB kills one child every 5 minutes. In countries with high burden TB, it may be as high as 20– 40 % of all new TB cases. More than 74,000 children die from the disease each year. Of the one million estimated cases of TB in children worldwide, 75 % occur in the 30 high-burden countries. As childhood TB reflects ongoing transmission in the community, children are affected most acutely in areas where adult TB is poorly controlled. Childhood TB is usually acquired from an infectious adult contact [2].

The incidence and prevalence of pediatric tuberculosis (TB) worldwide varies significantly according to the burden of the disease in different countries. It has been estimated that 3.1 million children under 15 years of age are infected with TB worldwide. According to the World Health Organization (WHO), children with TB represent 10 % to 20 % of all TB cases. The majority of these cases occur in low income countries where the prevalence of (HIV/AIDS) is high. TB occurs frequently among populations, such as malnourished individuals, and those living in crowded areas.

Pulmonary TB in children can range from an asymptomatic primary infection to a progressive primary TB. Primary TB is very often characterized by the absence of signs on clinical evaluation. Asymptomatic presentations are more common among school-age children (80-90 %) than in infants less than one year old (40-50 %) [3].

Extra pulmonary TB occurs more commonly in immunosuppressed persons and young children. In those with HIV this occurs in more than 50% of cases. Notable extra pulmonary infection sites include the pleura (in tuberculosis pleurisy), the central nervous system (in tuberculosis meningitis), the lymphatic system (in scrofula of the neck), the genitourinary system (in urogenital tuberculosis), and the bones and joints (osseous tuberculosis). Tuberculosis may become a chronic illness and cause extensive scarring in the upper lobes of the lungs [4].

Tuberculosis [TB] remains major Public health problem and among the top ten leading causes of human deaths worldwide. In 2016, alone 10.4 million new cases and 1.5 million deaths were reported globally. Its impact was more intense in developing countries where limited resource and integrated health care service. In this under resourced areas prevalence were high Particularly in south East Asian and western pacific regions with proportion of 58% share from total global estimate of New TB cases; and Africa continent were home for 28% of the global new TB cases, including the most sever Burden of TB with 281 cases for every 1000,000 people, which is more than double the global average of 133 cases per 100,000 populations [5].

Tuberculosis (TB) can involve any organ system in the body. While pulmonary TB (PTB) is the most common presentation, extra PTB (EPTB) is also an important clinical problem. EPTB involve organs such as pleura, lymph nodes, abdomen, genito-urinary tract, skin, joints, bones, tubercular meningitis, tuberculoma of the brain, etc. The problem of EPTB is still high, both in developing and developed countries. A diagnosis of extra pulmonary tuberculosis is generally made with the aid of several diagnostic tests. Depending on the site of infection, individuals may undergo general tests that include a blood panel and urinalysis. Imaging tests may be conducted to check for signs of inflammation, as often occurs in the presence of infection, and evaluate soft tissue and organ condition and functionality. Aspiration may be performed, which involves the use of a needle to obtain a fluid sample, if infection is suspected in one's joints or membranous tissues, such as the pericardium that protects the heart [6].

Aside from HIV infection, there are other contributory factors that may increase one's chance for infection. Those who have knowingly been exposed to the tuberculosis pathogen, but have never received a diagnosis, are considered at greatest risk for developing disseminated TB. Certain chronic conditions, such as diabetes and alcoholism, which can impair one's immunity, may also place an individual at significant risk for infection and complications [7].

Tuberculosis (TB) lymphadenitis is the most common presentation of EPTB and has been shown in about 35% of EPTB cases. TB meningitis is the most devastating form of meningitis and occurs in 7–12% of TB patients in developing. Osteoarticular TB accounts for about 1–3% of all TB cases and is the major cause of osteomyelitis .Any bone, joint or bursa can be infected but the

spine, hip and knee are the preferred sites of infection, representing 70–80% of the infections. Genitourinary TB comprising of genital and renal TB is the second most common EPTB and contributes up to 46% cases of EPTB while abdominal TB contributes up to 10–12% of EPTB cases, and much increase in this disease is because of HIV pandemic [8].

Various methods are employed for the diagnosis of EPTB such as smear microscopy, culture identification, and histopathology and nucleic acid amplification (NAA) tests.

In 2014, WHO has recommended Xpert over the conventional tests (including conventional microscopy, culture or histopathology) for testing specific non-respiratory specimens from presumptive EPTB patients However, this was a conditional recommendation due to very low-quality evidence available. More studies are therefore needed particularly in settings with high EPTB prevalence [9].

Tuberculosis in children has unique features, different from adults, which make the diagnosis more difficult. The symptoms of TB in children have a broad spectrum changing from non-specific symptoms to severe clinical presentations. Although pulmonary involvement is frequent, all organs can be involved [10].

The current study will utilize Genxpert MTB/RIF assay and MGIT by involving verities of Specimens like Pleural fluid, Gastric aspirate, CSF, Synovial fluid, Pus, Peritoneal, Ascitic fluid, lymph node aspiration was used as a diagnostic input for diagnosis of extra pulmonary tuberculosis in those under fifteen children.

1.2 Statement of the problem

Tuberculosis (TB) is a leading infectious cause of death worldwide, with an estimated 1.8 million deaths in 2015; it also remains the leading cause of death in people living with HIV [11].
Tuberculosis (TB) remains a major global health problem. It causes ill-health among millions of people each year and ranks in the top ten leading causes of mortality. About a third of the world's population is estimated to be infected with TB bacilli and hence at risk of developing active disease.

Seventy-five percent of TB in children occurs in 30 high incidence countries largely in Sub-Saharan Africa and Asia..TB caused illness in nine million people and 1.5 million deaths globally. Half a million children become ill with TB every year. There are 10 million children worldwide who had been orphaned because a parent died of TB. There were an estimated 10.4 million new cases of TB disease (also known as active TB) in 2015 and this included 1.2 million among HIV positive people. There were an estimated 3.5 million cases among women. There were also an estimated 1.0 million cases of TB in children [12].

Confirming the diagnosis of childhood tuberculosis is a major challenge. Non-standardized entry criteria for case enrollment, difficulty with expectorating sputum, the lower bacillary load and limited culture and DST testing laboratories could hinder determining the true burden and the drug profile. Thus, drug profile studies in children are required to determine the actual prevalence of drug resistant TB in children. The diagnosis of EPTB is often difficult to establish, considering that number of bacteria in specimens is often very low, a collection often requires invasive procedures, and it is not easy to obtain multiple samples. It is also not given priority in TB control programs in developing countries as the proportion is low and less infectious than PTB [13-14].

The impact of the HIV pandemic on children has been huge, and children account for around 10% of new global HIV infections with an estimated 3.4 million children aged less than 15 years living with HIV [15].

Children with TB are given low priority in most national health programs and are neglected in this epidemic. Recent technological advancements in diagnosis of TB in adults have not been validated in children [16].

The WHO report states that "progress towards targets for diagnosis and treatment of MDR TB is far off-track," with less than 25% of MDR TB cases detected in most MDR TB-burdened countries .The estimated TB cases and TB deaths in children were 6% and 8% of the global totals, respectively, in 2012 [17].

Ethiopia ranks eighth in the list of the 30 high TB burden countries which accounts for about 80% of the world's TB cases. According to the Global TB report 2013, there were an estimated 230,000 (247 per 100,000 populations).

Ethiopia had one of the lowest estimated rates of MDR TB in both new and retreatment cases (1.6% and 12%, resp.) among 27 high-burden countries. This report also showed that only 1% of new bacteriologic ally confirmed TB cases and only 4.4% of retreatment cases had DST coverage [18]

Investigation of DR-TB and their drug profile in children is limited, largely due to the well-known difficulties to isolate M. tuberculosis from pediatric specimens and very limited DST testing laboratories. The diagnosis of EPTB in children is often difficult for establishing, considering that number of bacteria in specimens is often very low, a collection often requires invasive procedures, and it is not easy to obtain duplicated samples. It is also not given priority in TB control programs in developing countries as the proportion is low and less infectious than PTB. There could be also an emerging problem of drug resistance in EPTB individuals and particularly in those individuals co infected with HIV. MDR-TB and XDR-TB (extensively drug resistant TB) are two crucial forms of drug resistance. RIFF resistance is used as a surrogate marker for uncovering MDR as > 90% RIF resistant isolates are also ionized [19].

In the circumstance of assessment to rapidly diagnose and treat the affected children leads to increased morbidity and mortality, development of secondary resistance (including extensively drug-resistant tuberculosis) and ongoing transmission of the disease. In this condition, not only rapid TB case detection, but also the early detection of MDR status is important. Conventionally the diagnosis of pulmonary tuberculosis has been based on clinical findings, chest X-ray findings, smear microscopy for acid fast bacillus, or bacterial isolation by culture [20].

The current molecular diagnostic techniques are increasingly high sensitivity and specificity. The World Health Organization (WHO) has endorsed the implementation of Gene Xpert MTB/ RIF assay for national tuberculosis programs in developing countries. The Xpert MTB/RIF is an automated, user friendly and rapid test based on nested real-time PCR assay and molecular

technology for MTB detection and RIF resistance. The results are obtained within a short period of 2 hours. Further on, the technique is not prone to cross-contamination, requires minimal biosafety facilities and has a high sensitivity in smear-negative extra pulmonary TB [21].

Young children are rarely able to expectorate sputum; therefore, other respiratory samples, such as gastric aspirates (GA) or bronchoalveolar lavages (BAL), can be obtained for diagnostic purposes, although these procedures are more unpleasant for a child than sputum collection. Collection of non-respiratory samples (lymph node (LN), pus or tissue biopsy) is necessary to diagnose extra pulmonary TB; however, these procedures are relatively invasive. Diagnosis of tuberculosis (TB) in children is challenging due to insufficient specimen material and the scarcity of bacilli in specimens.

1.3 Significance of the study

The current global drive to eliminate tuberculosis has increased the focus on childhood TB and it has now become crucial to implement the WHO recommendations especially in resource-poor high TB burden countries.

This study will provide information on the problem of EPTB in the listed specific age group. This study will also help health care workers (Physicians) to strength the evaluation of the presumptive TB patients other than pulmonary and will increase the trend of utilization of molecular test for diagnosis of TB that involves different body parts.
The study was more helpful for policy makers in the case of developing, updating and establishing of guidelines .It may also include in the eligibility criteria of Genxpert utilization, Since using this technique increases the detection of TB from different types of body fluid specimens .It will also helps public health concern and activities so as to decline cases of mortality and morbidity.

Moreover, this study will be used as a resource for those researchers in order to compare and contrast for TB case detection with other techniques so that it will be more crucial in avoiding or minimizing the community health related issues regarding to TB infection and related co-infections.

This study will also provide crucial information, awareness and consideration on usage and processing of: small volume of samples for a given specimen type, incomplete information on HIV status, limited data for assessing the accuracy of Xpert®MTB/RIF for detection of rifa mpicinresistance, and considerable differences in the preparation of specimens for testing

2 Literature Review

A study conducted at Queensland Mycobacterium Reference Laboratory (QMRL) Australia as from 2012 and 2014.Out of 269 samples 100 of them were children EPTB. One hundred and ninety of the 269 samples analyzed were GeneXpert MTB negative, of which nine grew MTB, 86 grew NTM, 94 were culture negative and one sample was contaminated. Seventy-nine of the 269 samples analyzed were GeneXpert TB positive. Seventy of the 79 samples grew MTB and nine were culture negative [22].

Study conducted in Kosovo University showed that Of the 116 specimens investigated, 28 (24.1%) were MTBC-positive by culture, while 34 (29.3%) were positive by Xpert assay. Two samples showed false-negative Xpert results. Compared with culture, the Xpert assay achieved 82.3% (95% CI: 65.5%–93.2%) sensitivity, and 97.6% (95% CI: 91.5%–99.7%) specificity. GeneXpert could detect 11.7% and 50% additional positive cases as compared to LowensteinJensen culture and smear microscopy, respectively. Three cases with resistance to rifampin were detected from clinical isolates [23].

Similar study conducted in Beijing Pediatric Research Institute A total of 163 suspected UTB patients were consecutively enrolled in the analysis, including 37 (22.7%) culture-positive and 44 (27.0%) clinically diagnosed UTB cases. Compared with conventional culture, the sensitivity of GeneXpert (94.6%) was significantly higher than that of smear microscopy (40.5%, $P < 0.001$). When setting clinical diagnosis as gold standard, 51 out of 81 clinically diagnosed UTB cases were detected by GeneXpert, demonstrating a sensitivity of 63.0%, which was significantly higher than that of smear microscopy (18.5%, $P < 0.001$) and culture (45.7%, $P = 0.027$), respectively [24].

Study conducted in Pakistan M. tuberculosis (MTB) were detected by Xpert MTB/RIF test in 111 (45.3%) out of 245 samples. Of these, 85 (34.7%) were smear positive on ZN staining and 102 (41.6%) were positive on LJ cultures. Rifampicin resistance was detected in 16 (6.5%) patients. Nine out of 19 pus samples (47.3%) were positive for MTB by Gene Xpert, 03 (15.8%) on ZN staining and 04 (21%) on LJ culture [25].

Study conducted in India showed Out of 30 lymph node samples studied, AFB smear was found positive only in 2 specimens and both of these were culture and GeneXpert positive. Out of the 28 smear negative, 15 were culture positive, out of which 14 were also GeneXpert positive. Out of the 13 culture negative samples, 9 were found to be positive on GeneXpert [26].

A study conducted in Israel among immigrants from High to low tuberculosis endemic countries showed that out of 479 children both culture and Genxpert confirmed EPTB extra thoracic lymphadenitis (12.5),synovial fluid (3.6%) ,pleural fluid (1.3%),CSF (1%) ,and ascetic fluid is also (1%) [27].

Another study conducted in Germany on 521 specimens, all of which were sent to the National Reference Center for Mycobacteria between May 2009 and August 2010. Among the 245 tissue samples, the majorities were lymph node specimens, but they also included skin, kidney, spleen, liver, bone, and lesion specimens. Overall, 62 (11.9%) of the 521 specimens tested were positive for mycobacteria by culture. Out of these 62 positive cultures, 6 (9.7%) were also smear positive for acid-fast bacilli. Thirty MTBC and 17 NTM strains could be isolated from the tissue specimens, and 8, 5, and 2 MTBC strains were isolated from gastric fluid, urine, and stool specimens, respectively [28].

Another study conducted in Tunisia showed 59 patients out of 153 presented with tuberculosis. PCR was positive in 50 samples and all of these samples were susceptible to rifampicin. Sensitivity, specificity, positive predictive value, and negative predictive value of the GeneXpert® test were 84.7%, 96.8%, 94.3%, and 91%, respectively, compared with diagnosis. There was a statistically significant difference between the direct test and the GeneXpert test, and between culture and the GeneXpert test. No statistically significant difference was observed between pathological results and the GeneXpert test. Sensitivity of the GeneXpert test was 87.5% in biopsies, 80% in pus and abscesses, and 66.7% in biological fluids. All strains were susceptible to rifampicin with culture and GeneXpert® test [29].

A study conducted in South Africa on total of 260 with recruitment of 3640 childrens investigated from 2013 to 2015 for extrapulmonarytuberculosis .Culture tests were positive for

tuberculosis in 12% (420 0f 3640) of all children assigned and Genxpert was positive in 11% (406 of 3640).Compared with culture the pooled sensitivity and specificity of Genxpert for tuberculosis detection was 62% (95 % credible interval 51-73) and 98% (97-99) respectively [30].

An institutional based study was conducted among EPTB suspected patients at the University of Gondar Hospital. A total of 141 extra pulmonary suspected patients were enrolled in this study. The overall Prevalence of culture confirmed extra pulmonary tuberculosis infection was 29.8%, but the Gene Xpert result showed a 26.2% prevalence of Mycobacterium tuberculosis complex infection. The 78.4% prevalence of extra pulmonary tuberculosis infection was found to be higher among the adult population. The prevalence of HIV infection among EPTB suspected patients was 14.1%, while it was 32.4% among Gene Xpert-confirmed extra pulmonary TB cases (12/37). Tuberculosis lymphadenitis was the predominant (78.4%) type of EPTB infection followed by tuberculosis cold abscess (10.7%) [31].

Another cross-sectional study was conducted in patients with presumptive TB from 1st April 26 2015 to 30th August 2016 in Gambo Hospital, Ethiopia on 309 unique patients; 197 (63.8%) were less than 14 years old, and 165 (53.4%) were male. The most commonly analyzed sample was gastric aspiration (n=144, 46.6%) followed by sputum (n=92, 29.8%). Gastric aspiration was performed mainly in children (98.6%, 142/144; $p<0.001$), while peritoneal effusion (94.4%, 17/18; $p<0.001$), pleural effusion (80.8%, 21/26; $p<0.001$), lymph node (63.6%, 14/22; $p=0.01$), and sputum (56/92, 60.9%; $p<0.001$) were performed mainly in adults. The results of Xpert MTB/RIF were positive in 76.2% (16/21) of the lymph node samples ($p<0.001$), 22.3% of the gastric aspiration samples, 1.5% (1/17) of the asciatic fluid samples, and 0.0% (0/25) of the pleural effusions ($p=0.002$) [32].

2.1. Risk factors for extra pulmonary Tuberculosis

Children are at higher risk of contracting TB infection and disease. Studies have shown that 60–80% exposed to sputum smear-positive case became infected compared to only 30–40% who are exposed to a sputum smear-negative source case. Majority of the children less than 2 years of age get infected from the household source case, whereas, with children more than 2 years of age, majority of them became infected in the community. Household sputum positive source case

is the single most important risk factor for children and remained an important contributor to infection up to 5–10 years of age .Most of the disease manifestations develop within the first year following primary infection, identifying the first year following exposure as the time period of greatest risk. Children with primary infection before 2 years or after 10 years of age were at increased risk for disease development .The highest risk for TB-related mortality following primary infection occurred during infancy. The risk declined to 1% between 1 and 4 years of age, before rising to more than 2% from 15 to 25 years of age .These findings provided the scientific basis for classical contact investigation practices, which focus on children less than 5 years of age in most developing countries and all household contacts in most industrialized countries [33].

.Extra pulmonary tuberculosis (EPTB) appears to be a marker of an underlying immune defect. The risk of extra pulmonary disease is increased in HIV-infected persons; it occurs in 10% to 20% of HIV-seronegative persons but in 40% to 80% of those infected with HIV. The increased risk in HIV-infected persons has been associated with advanced immune suppression. Thus, if there is a predisposition to developing tuberculosis, the immunologic defects associated with this predisposition should be most readily identified among persons with EPTB disease .The risk of progression from exposure to the tuberculosis bacilli to the development of active disease is a two-stage process governed by both exogenous and endogenous risk factors. Exogenous factors play a key role in accentuating the progression from exposure to infection among which the bacillary load in the sputum and the proximity of an individual to an infectious TB case are key factors. Similarly endogenous factors lead in progression from infection to active TB disease. Along with well-established risk factors (such as human immunodeficiency virus (HIV), malnutrition, and young age), emerging variables such as diabetes, indoor air pollution, alcohol, use of immunosuppressive drugs, and tobacco smoke play a significant role at both the individual and population level. Socioeconomic and behavioral factors are also shown to increase the susceptibility to infection. Specific groups such as health care workers and indigenous population are also at an increased risk of TB infection and disease. This paper summarizes these factors along with health system issues such as the effects of delay in diagnosis of TB in the transmission of the bacilli [34].

2.2 Transmission & Pathogenesis

TB is caused by an organism called *Mycobacterium tuberculosis* that is spread from person to person through the air. *M. tuberculosis* organisms are sometimes called tubercle bacilli. When a person with infectious TB disease coughs or sneezes, droplet nuclei containing tubercle bacilli may be expelled into the air. Other people may inhale the air containing these droplet nuclei and become infected.

TB infection begins when the tubercle bacilli multiply in the small air sacs of the lungs. A small number enter the bloodstream and spread throughout the body, but the body's immune system usually keeps the bacilli under control. People who have latent TB infection (LTBI) but not TB disease do not have symptoms of TB, and they cannot spread TB to others.

In some people who have LTBI, the immune system cannot keep the tubercle bacilli under control and the bacilli begin to multiply rapidly, causing TB disease. This can happen very soon after TB infection or many years after infection. About 10% of people who have LTBI will develop disease at some point, but the risk is greatest in the first year or two after infection, than for other people.

TB disease usually occurs in the lungs (pulmonary TB), but it can also occur in other places in the body (extra pulmonary TB). Miliary TB occurs when tubercle bacilli enter the bloodstream and are carried to all parts of the body, where they grow and cause disease in multiple sites

Pathogenesis

When droplet nuclei are inhaled, most of the larger particles become lodged in the upper respiratory tract, where infection is unlikely to develop. However, smaller droplet nuclei containing the tubercle bacilli may reach the alveoli, where infection begins.

The tubercle bacilli that reach the alveoli are ingested by alveolar macrophages; the majority of these bacilli are destroyed or inhibited. A small number multiply intracellular and are released when the macrophages die. These bacilli can spread through the lymphatic channels to regional lymph nodes and then through the bloodstream to more distant tissues and organs, including areas in which TB disease is most likely to develop: the apices of the lungs, the kidneys, the brain, and bone. Extracellular bacilli attract macrophages from the bloodstream. The immune response kills most of the bacilli, leading to the formation of a granuloma. At this point the person has TB infection, which can be detected by using the tuberculin skin test. It may take 2-10 weeks for the infected person to develop a positive reaction to the tuberculin skin test. Immune responses soon develop to kill the bacilli. Within 2 to 10 weeks after infection, the immune system is usually able to decline the multiplication of the tubercle bacilli, preventing further spread.

Persons who are infected with *M. tuberculosis,* but who do not have TB disease cannot spread the infection to other people. TB infection in a person who does not have TB disease is not considered a case of TB and is often referred to as latent TB infection (LTBI).

In some people, TB bacilli overcome the defenses of the immune system and begin to multiply, resulting in the progression from TB infection to TB disease. This process may occur soon after or many years after infection. In the United States, unless they are treated, approximately 5% of persons who have been infected with *M. tuberculosis* will develop TB disease in the first year or two after infection and another 5% will develop disease sometime later in life. Recent infection (within the past 2 years) with *M. tuberculosis* is therefore an important risk factor for progression to TB disease. In all, in approximately 10% of persons with normal immune systems who are infected with *M. tuberculosis,* TB disease will develop at some point [34-35].

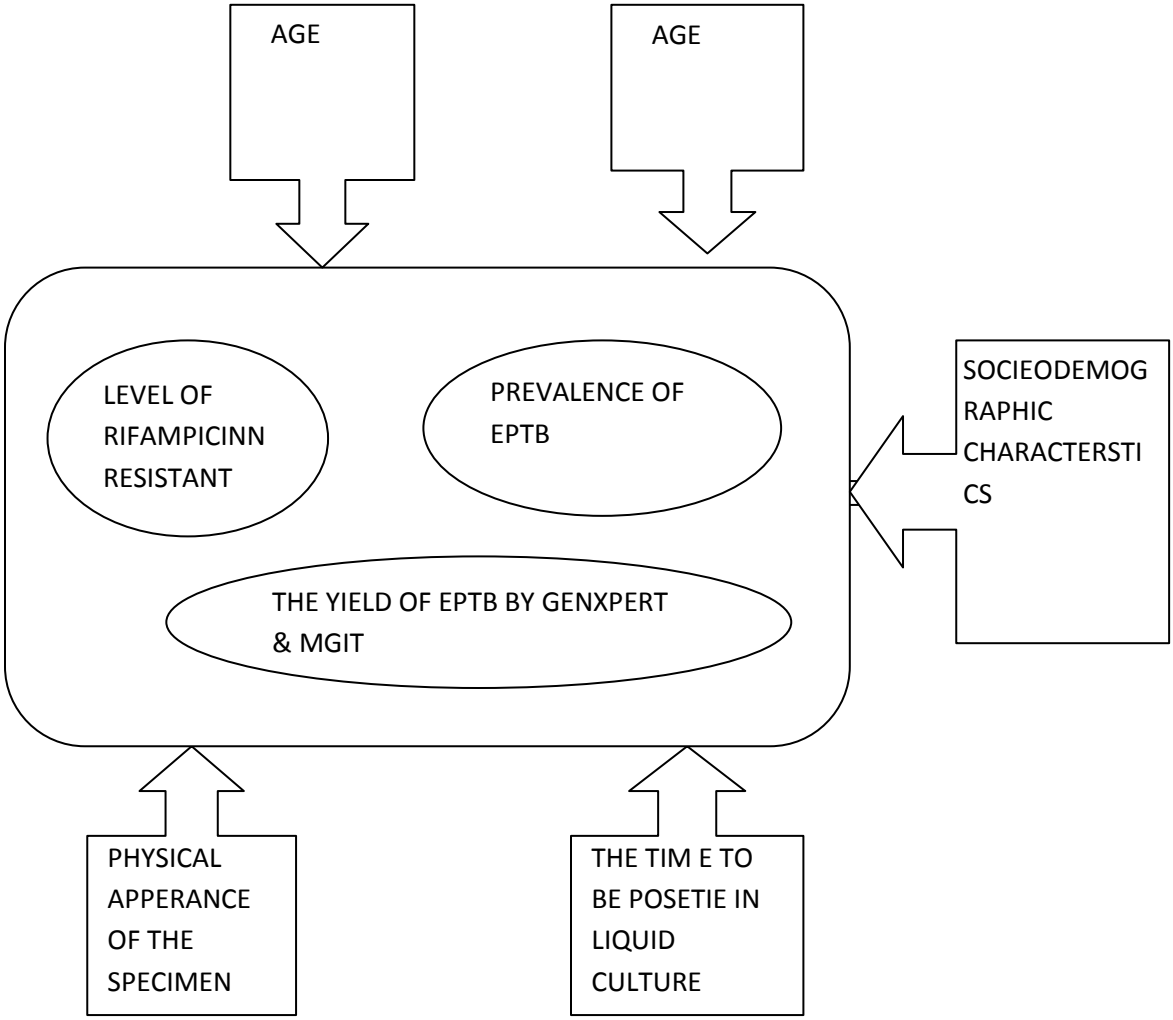

Figure 1. Conceptual frame work

3 Objectives

3.1 General objectives

- To determine the prevalence of *Extra pulmonary tuberculosis and rifampicin resistance in children at selected Health facilities of South west shewa zone, Oromia*.

3.2 Specific objectives

- To determine the prevalence of extra pulmonary tuberculosis Using Genxpert assay and Confirming using MGIT/LJ technique
- To determine the yield of Gene xpert assay method and MGIT
- To assess associated risks factors for EPTB

4 Hypothesis

Regarding to Tb from gastric aspiration there was no difference ,but regarding with Tb from lymphadenitis, synovial ,ascetic, pleural, between the current study and a research done in Rural Health Facilities in Southern Ethiopia (Gambo Hospital) showing the burden of EPTB in children.

5 Materials and methods

5.1 Study area

The study was conducted in St.Luke Catholic Hospital ,Tullubolo Hospital, Atat Hospital, Ameya Hospital, Woliso 1&2 HC, Dilela HC South west Shewa, Oromia. St.Luke Catholic Hospital is an NGO Hospital found in South West Shwea Zone with bed capacity of 350. Atat, have bed capacity of 220, Tullubollo with bed capacity of 200 catchment population of about 4 million people both st.luke and tullubolo hospital where as atat hospital do have 2 million people.

Figure-2 Map of south west shewa, that were indicate the study site May, 2018

5.2 Study period

The study was conducted from December 20, 2017 to April 26, 2018 in South West Shewa Zone, Oromia, Ethiopia.

5.3 Study design

A cross sectional study was conducted from December 20, 2017 to April 26, 2018, among all presumptive EPTB and those who fulfill the inclusion criteria. requested for Gene xpert in South West Shewa Zone, Oromia.

5.3.1 Source population

All presumptive TB patients who had visited in the health facilities .during the study period was considered as source population.

5.3.2 Study population

All presumptive children who were suspected to be EPTB patients requested for Gen xpertin the health facilities during the study period and fulfill the inclusion criteria were eligible to be enrolled in the study.

5.4 Inclusion and Exclusion criteria

5.4.1 Inclusion criteria

Extra pulmonary tuberculosis suspected patients willing to participate in the study were included.

5.4.2 Exclusion criteria

- Previously known extra pulmonary tuberculosis patients who are on anti-TB treatment
- Previously confirmed pulmonary tuberculosis (PTB) cases.

5.5 Study variables

5.5.1 Dependent variables

- Level of rifampicin resistance
- Prevalence of EPTB
- The yield of EPTB by gene xpert& MGIT / LJ

5.5.2 Independent variables

- Age, sex, Socio-demographic
- The time to test positivity in liquid culture.
- Physical appearance of the specimens

5.6 Measurement and data collection

5.6.1 Sample size determination

The sample size is determined using the following single population proportion formula:
N = z2 p (1-p)/ w2, where N = the number of EPTB suspected patients; Z = Standard normal Distribution value at 95% CI which is 1.96; P = the prevalence of extra pulmonary tuberculosis Infection in under fifteen children is = 22.3% [32].W= the margin of error taken as 4% with 10% contingency sample i.e. 416 x 0.1=41.6.Accordingly, a total of 458 samples was collected from different body sites of EPTB suspected children.

5.6.2 Sampling methods

Convenient sampling technique was applied on the Samples that was collected from presumptive extra pulmonary tuberculosis patients who will come to St.Luke catholic hospital and Tullu bollo hospital consecutively until the required number of patients will offer the specimens.

5.7 Data collection Tool and procedure

5.7.1 Data collection Tool

By using structured data collection questionnaire customized from national gene xpert request paper according to study objective besides questionnaire which was prepared by principal investigator.

5.7.2 Sample collection and analysis procedure

Extra pulmonary specimens was collected from the patients who visits St.Luke catholic hospital &Tullubolo Hospital ,Atat Hospital ,Ameya hospital and woliso,Dilela HC during the study period by the requesting physicians and experienced senior nurse. During sample collection SOPS for sample collection and transportation was used. The fluid is aspirated with syringe and needle from infected organ in sterile condition. The air is compressed out and it was transferred

to falcon tube for gene xpert and Culture the other type of specimen is Gastric lavge that was collected with the help of Physicians using NG tube and it was transferred in to the falcon tube .The sample was refrigerated even if the process was on site sample collection the duplicated sample were refrigerated and send for culture and sensitivity test. The sample was transported to microbiology laboratory of St.luke hospital and analyzed by gene xpert immediately as protocol states. .

5.7.3 Principle and procedure of Gene xpert

Body fluid samples were diluted in 2:1 as protocol states then the diluted samples will be inoculated at room temperature for about 15 minutes .Then the diluted specimen about 2 ml will be transferred to the Genxpert Cat ridge, it will be loaded in to the Genxpert assay after 2 hour the result will be displayed on the computer as MTB detected (Positive0 or MTB not detected 9negative) if the specimen contains MTB resistant it will also display the result on the computer. [35].

5.7.4 Principle of MGIT 960

The EPTB samples will be decontaminated with (NALC-NAOH) and centrifuged and about 0.5 ml decontaminated sample will be placed in to the MGIT tube which contains 7ml middle brook broath base media,OADC as enrichment will be added and the reconstituted PANTA as suppresser of contamination, oxygen-quenched florochrome then it will be loaded in to BACTEC MGIT 960 system at 37 degrecentigrade and if MTBC is found then the BACTEC MGIT 960 system will detect the flourished light since oxygen is depleted then samples will be taken from MGIT tube and cheeked for AFB and blood agar if the bacteria grows on blood agar it is contaminated and then the specimen will be discarded and if AFB is positive and no growth on blood agar we get MTBC . By using capillaNeo test immunocromatographic test MTBC will be differentiated from non mycobacterium tuberculosis. [36].

5.7.5 Drug sensitivity Test

Based on inoculating one or more dilutions of cultured mycobacteria on drug-free media and on media containing antimycobacterial agents. The proportion method permits quantification of the proportion of drug resistant bacilli in the clinical isolate by the comparison of the number of bacterial colonies growing on the drug free control and drug containing media. Drug resistance is

generally considered to be present when the growth on the drug-containing medium is more than 1% of the growth in a drug free media (the control growth) [37].

5.8 Data quality assurance

Data quality was ensured through the use of standardized data collection tools, proper training before the start of data collection and intensive supervision during data collection by the principal investigator. For laboratory analysis Pre-analytical, analytical and post-analytical stages of quality assurance that are incorporated in Standard operating procedures (SOPs) of the microbiology laboratory of St.Luke Catholic, Atat, Ameya Hospital and Tullubollo Hospital ,Woliso HC 1,Woliso HC2,Dilela HC was strictly followed. Besides, well-trained and experienced laboratory professionals will participate in the laboratory analysis procedure.

5.9 Data analysis and interpretation

Data was entered into Microsoft excel and exported to the Statistical package for Social Science (SPSS) version 20.0 for analysis. Descriptive analysis was used to determine demographic and clinical characteristic.

5.10 Operational Definition

EPTB: is defined as tuberculosis that occurs and found outside the lung

MDRTB: Is a form of tuberculosis (TB) infection caused by bacteria that are resistant to treatment with at least two the most powerful first line anti-TB medications (Drugs), Isonizid& Rifampin

5.11 Ethical Consideration

Ethical clearance was collected from the research and ethics review committee of the department of medical laboratory sciences, school of allied health science, college of public health, Addis Ababa University. The participants after reading the consent form if they agree they were involved on the study. Concerning the confidentiality of the result, since all clients who are participated on the study have unique ID number confidentiality was kept throughout the study**.**

5.12 Dissemination of results

The study report was submitted to AAU, College of Health Science, and School of Allied Health Science.

6 Results

Sociodemographic characteristics and types of specimens

A total of416 EPTB suspected children age ranged from 21 days to 14 years (Mean=6.7 & 4.1+SD) were included in this study and about 45.9 %(191/416) were children less than five years of age 31.5 %(131/416) from 6-10 years, and 22.6 %(94/416) were from 11-14 years of age. Participants 48.8 %(203/416) were male and 51.2 %(213/416) females. About 59.1 %(246/416) of the study participants were living in rural area and 40.9 %(170/416) were in urban area. More than half 55.05% (229/416) participants were attending primary school, 44.9 %(187/416) had no formal education.

Overall a total of 416 presumptive EPTB samples were collected from different body sites of the study participants' .From these samples 28.6%(119/416) were gastric aspirate, 15.9 %(66/416) pleural fluid ,8.4 %(35/416) CSF, 6.5 %(27/416) synovial fluid, 14.2%(59/416) pus ,21.9%(91/416) peritoneal fluid, 1.92 %(8/416) lymph node aspirate, 2.4 % (10/416) were accounted for ascetic fluid

All presumptive participants were new and few participants were presumptive MDR-TB 0.96 %(4/416) of 416 study samples 4.8 %(n=20/416) were positive for HIV test. **"Table 1".**

Table 6.1 Socio demographic characteristics of study participants in selected health facilities of south west shewa, oromia, Ethiopia from December 20, 2017- April 26 2018

Variables		Frequency	Percentage%
Age	**0-5 Year**	**191**	**45. 92%**
	6-10 Year	**131**	**31.5%**
	11-14 Year	**94**	**22.6%**
Gender	**Male**	**203**	**48.8%**
	Female	**213**	**51.2%**
Residence	**Urban**	**170**	**40.9%**
	Rural	**246**	**59.1%**
Educational status	**No formal education**	**187**	**45 %**
	Primary	**229**	**55 %**
Site of specimen	**Pleural**	**66**	**15.9%**

	Gastric aspirate	**119**	**28. 6%**
	CSF	**35**	**8.4%**
	Pus	**59**	**14. 2%**
	Peritoneal fluid	**91**	**21.9%**
	Lymph node aspirate	**08**	**1.9%**
	Ascitic fluid	**10**	**2.4%**
	Synovial fluid	**27**	**6.5%**
Type of TB	**Presumptive TB**	**412**	**99 %**
	MDR-TB	**04**	**1%**
HIV Status	**Positive**	**20**	**4.807%**
	Negative	**396**	**95. 2%**

Table 6.2 Physical characteristics of body fluid sample among the study participants in selected health facilities of south west shewa, oromia, Ethiopia from December 20, 2017- April 26 2018

Parameter		**Frequency**	**Percentage**
Sample appearance	**Clear**	**229**	**55%**
	Turbid	**33**	**7.9%**
	Muccoid	**153**	**36. 8%**
	Bloody	**0**	**0**

Physical assessment of body fluid samples showed that 55% (229/416) were clear and 7.9 %(33/416) were turbid, 36.8 %(153/4160 were muccoid.

Table 6.3 Bacterial load of extra pulmonary tuberculosis using Genxpert MTB/RIF assay among the study participants in selected health facilities of south west shewa, oromia, Ethiopia from December 20, 2017- April 26 2018

Bacterial load using Xpert MTB/RIFF assay	**Positive**
Medium (16<ct>22)	**4**
Low (22<ct>28)	**21**
Very low (28<ct>38)	**41**
Total	**66**

We have compared the yield of EPTB using Genxpert about 15.8 % (66/416) of body fluid samples were positive by XpertMTB/RIFF assay with 4 medium, 21 low and 41 very low bacterial load. Among the 66 samples in which it was positive by Genxpert 4 samples have got bacterial load of medium in which the ct was between 16 and 22, 21 specimens were also got bacterial load of low which have ct between 22 and 28 and lastly majority of the specimens were between 28 and 38 ct in which the Genxpert system terminates at ct of 38

Table.6.4 Yield of extra pulmonary using Genxpert MTB/RIFF assay and MGIT among the study participants at selected health facilities of south west shewa, oromia, Ethiopia from December 20, 2017- April 26 2018

	Positive	**Negative**	**Total**
Genxpert	**66**	**350**	**416**
Culture growth	**43**	**344**	**397**
Contaminated	**19**		

We have got Xpert positive result 66 out of 416 study participants and culture growth of 43 from the total of tests 19 of the specimens were contaminated and they were excluded from the analysis.

Table 6.5 Gene Xpert detection rate and associated factor among among the study participants at selected health facilities of south west shewa .oromia, Ethiopia from December 20,2017to April 26 2018

Variables		Genxpert		COR (95% CI)	P-Value	AOR	P-Value
		Detected (%)	Not Detected (%)				
Age	0-5 Year	149(78%)	41(22%)	1.00	----		
	6-10 Year	115(87.8%)	16(12.2%)	2.662(0.392, 6.250)	0.012	1.85(0.951, 3.617	0.07
	11-14 Year	85(90.4%)	9(9.57%)	1.314(0.441, 0.384)	0.535		
Gender	Male	174(85.7%)	29(14.3%)	1.00	--		
	Female	175(82.6%)	37(17.4%)	0.768(0.269, 0.989)	0.325		
Residence	Urban	140(82.3%)	30(17.6%)	1.00	---		
	Rural	209(84.9%)	37(15.4%)	0.826(0.269, 0.505)	0.478		
Educational status	No formal education	148(79.1%)	39(20.8%)	0.529(0.0270, 5.560)	0.018	0.873(0.336, 2.269)	0.078
	Primary	201(87.8%)	27(11.8%)	1.00	--		
Types of TB (Clinical)	Presumptive TB	345(83.7%)	66(16%)	0.000(1.005, 0.000)	0.999		
	MDR-TB	4(100%)	0(%)	1.00	---		
Sero status	Positive	14(70%)	6(30%)	2.354(0.507, 2.846)	0.092		
	Negative	335(84.6%)	60(15.1%)	1.00	---		

Table 6.5.1 Gene Xpert detection rate among the study participants at selected health facilities of south west shewa .oromia, Ethiopia from December 20,2017to April 26 2018

		Genxpert detection		Total	P-value
		Detected	Nott detected		
Sample site	Peritoneal	0	91(100%)	91	0.004
	Gastric aspirate	28(23.53 %)	91(76.47%)	119	
	CSF	0	35(100%)	35	
	Pleural	3(4.54%)	63(45.45%)	66	
	Lymph node	8(100%)	0	8	
	Abscess	26(44.1%)	33(55.9%)	59	
	Synovial	1(3.7%)	26(96.3%)	27	
	Ascitic	0	10(100%)	10	

Table 6.6 Culture detection rate and associated factor among among the study participants at selected health facilities of south west shewa .oromia, Ethiopia from December 20,2017to April 26 2018

Variables		MGIT		COR (95%CI)	P-Value	AOR	P-Value
		Positive (%)	Negative (%)				
Age	0-5 Year	42(22%)	149(78%)	1.00			
	6-10 Year	8(6%)	123(94%)	8.550(0.612, 12.639	<0.001	4.878(1.781 , 13.365)	0.002
	11-14 Year	3(3%)	91(97%)	1.973(0.691, 0.967	0.325		
Gender	Male	16(7.8%)	187(92.1%)	1.00	--		
	Female	37(17%)	176(83%)	0.407(0.219, 0.758)	0.005	0.453(0.239 , 0.869)	0.016
Residence	Urban	22(13%)	148(87%)	1.00	--		
	Rural	31(12.6%)	215(87.4%)	0.970(0.540, 1.741)	0.919		
Educational status	No formal education	38(20.3%)	149(79.7%)	0.275(0.323, 15.983)	<0.001	0.568(0.176 , 1.832)	0.344
	Primary	15(6.5%)	214(93.4%)	1.00	--		
Types of TB (Clinical)	Presumptive TB	53(12.9%)	359(87.1%)	0.000(0.000, 1.005)	0.999		
	MDR-TB	0(%)	4(100%)	1.00	--		
Sero status	Positive	3(15%)	17(85%)	1.00	--		
	Negative	50(12.6%)	346(87.4%)	0.819(0.232, 2.895)	0.756		

Table 6.6.1 Culture detection rate among the study participants at selected health facilities of south west shewa .oromia, Ethiopia from December 20,2017to April 26 2018

		Culture detection rate		Total	P-value
		Positive	Negative		
Sample site	Peritoneal	0	91(100%)	91	0.001
	Gastric aspirate	30(25.2%)	89(74.78%)	119	
	CSF	1(2.85%)	34(97.14%)	35	
	Pleural	0	66(100%)	66	
	Lymph node	7(87.5%)	1(12.5%)	8	
	Abscess	14(23.73%)	45(76.27%)	59	
	Synovial	1(3.7%)	26(96.3%)	27	
	Ascitic	0	10(100%)	10	

Associated risk factors to EPTB

Socio-demographic characteristics such as residence, age and sero status were not significantly associated with extra pulmonary tuberculosis infections .Besides to this in the univarate model although statistically insignificant patients of age group 6-10 years were eight times more likely to develop EPTB disease than the others age group (COR=8.55 95%CI 2.575 ,28.387 **,P** <0.001 Age group of 11-14 years had less likely to develop EPTB than young age group (COR=1.973 ,CI 0.509,0.7.643 ,P 0.325 which were not significantly associated with EPTB infections .

On the other hand, HIV negative patients had less likely to develop EPTB than HIV positive one (COR=**2.354(0.507, 2.846), P (0.092)**

7 Discussion

Extra pulmonary tuberculosis is one of the highly prevalent diseases in developing countries including Ethiopia. The current study was to determine the prevalence of MTBC from different body fluids among presumptive extrapulmonarytuberculosis children. It is estimated that between 10 to 25% of tuberculosis infections occur in extra pulmonary sites worldwide .Now a day's extra pulmonary tuberculosis is becoming a major concern.

The overall prevalence of culture confirmed *Mycobacterium tuberculosis* complex infections among EPTB suspected cases in south west shewa zone, oromia region was 10.8% .The current study also showed that the prevalence of extra pulmonary tuberculosis infection among females 17 % which was higher than males 7.8 % the reason is unclear.

The age group of children with EPTB exposed of younger children with 0 -5 years being affected accounting 45.9% followed by 6-10 years old accounting 31.5% and 11-14 years old accounting about 22.6 % the least age group .In our study EPTB prevalence were almost the same with a study done by Tasbakan etal in Australia [22].

Genxpert positivity rate was 29.3% and culture positivity rate for MTBC were 24.1 % in contrast to our study in which Genxpert positivity rate was 15.8%, 13 samples were detected from culture negative samples .This could be due to the fact that the bacilli die due to different factors .On the other hand 9 samples were negative in Genxpert but positive in culture this might be due to limited bacilli found in the sample [23].

Other study conducted in Turkey by Dilber etal showed lowest prevalence of extra pulmonary tuberculosis sites with MGIT culture, 0.8% pleural fluid ,11.7% peritoneal fluid , CSF 0.8%, cold abscess 0.8% and comparatively highest prevalence with lymph node aspirate 11.1% in contrast to our study this could be due to study area, sample size [24].

Similar study done by Suleiman etal showed 47.3% positivity by Genxpert and 21% positive for MTBC by culture against to our study 15.8% Genxpert positive rate and 10.8% positive for MTBC by culture this could be due to different factors such as bacilli might die, high concentration of NAOH in decontamination process, generation of heat during centrifugation of the samples and high buffer PH [25].

TB lymphadenitis in our study was 1.92% which were comparatively very low in contrast to a study done in India by Sharma etal with the prevalence rate of culture confirmed 50% this could be due to the sample size, study area where awareness of TB was poor years ago [26].

In our study the overall extra pulmonary tuberculosis prevalence was a little bit higher than a study done by Denkinger etal in which culture confirmed and Genxpert positive rate were (12% vs 11%) this could be due to different factors that are contributing to the decline of TB infections [27].

Children living in rural areas had the highest prevalence of EPTB infection (12.6%) ,and (13%) of them were living in urban area those children living in rural areas might be exposed to inadequate nutrition which favors EPTB infections ,malnutrition which profoundly affects cell-mediated immunity which is a principal host defense against to TB besides to this malnutrition contributes to both mortality and morbidity due to tuberculosis infection .In this study gastric lavage tuberculosis was the most common (42.2%) .The overall prevalence in body fluids was 11.9% which was to the nearest similar to our study [28].

Similarly in our study pleural TB was (15.9%) which was almost the same to a study done in Israel by Daniel etal with culture and Genxpert confirmed prevalence of 15.5% [29].

Socio-demographic characteristics such as sex, residence, and educational status were not significantly associated with extra pulmonary tuberculosis infection which is similar with study in Gonder University [31].

Besides to this in the univarate model although statistically insignificant patients of age group 6-10 years were eight times more likely to develop EPTB disease than the others age group (COR=8.55 95%CI 2.575 ,28.387 ,P 0.000 Age group of 11-14 years had less likely to develop EPTB than young age group (COR=1.973 ,CI 0.509,0.7.643 ,P 0.325 which were not significantly associated(30) On the other hand, HIV negative patients had less likely to develop EPTB than HIV positive one (COR=2.354(0.507, 2.846), P (0.092)

Another institution based study conducted at university of Gondar by Baye etal showed that the overall prevalence Genxpert result was 26.2% and culture confirmed EPTB was 29.8% it was higher in contrast to our study this could be due to the size of the sample and the area of study (31). In our study Genxpert and cultured confirmed EPTB samples were 15.8% and 12.7% which is similar with a study done by Moure etal [30].

Varities of body fluids were useful samples to identify Mycobacterium tuberculosis in children in our study prevalence was 42.2% which was higher than a study done by Jose etal in southern Ethiopia with prevalence of 22.3% this could be due to children family, care givers with untreated, clinical contact and family history [32].

In our study prevalence of HIV in children were 4.8% which was much lower than a study done by Crofton etal which was 15.6% this could be due to delay in early diagnosis and treatment besides low expansion of ART coverage.

Tuberculosis and HIV/AIDS have a high synergistic effect on each other that one increases the progress of the other. Many scholars indicate that the presence of HIV infection increases the magnitude of the HIV infection. The immune deficiency syndrome due to the virus confers the dissemination of the bacilli form the primary site of infection, the lung to other body parts .In this study, the HIV status of EPTB suspected children was determined by rapid HIV test method. All Wanti Beijing positive samples were retested with unigold for confirmation and there were no discordant results.

The result of Genxpert and culture investigations showed a prevalence of 15.8% and 10.8% respectively .Discordant test result agreement was observed between Genxpert and culture .Taking culture as the gold standard. Culture methods were much more sensitive because fewer bacilli (10-100 bacilli/ml) can be detected.

In the present study unfortunately no sample were demonstrated MDR Mycobacterium tuberculosis complex infection among the Genxpert and Culture positive samples

The conventional culture (both LJ and MGIT) can take several weeks to months to yield detectable growth and DST result. It requires specialized laboratory facility including sophisticated bio-safety and highly trained laboratory personnel. TB culture is only available at national or reference laboratory level. It is also difficult to get access to these laboratory for the district level of the health system. Xpert MTB/RIF assay will represent an important contribution by providing MTB and RIF resistance results in a matter of hours. As observed in this study, it only took one day training to run the Xpert MTB/RIF assay. The turnaround time to get Xpert

result was one day. However, short expiry date of cartilage, constant supply of consumables and requirement of annual instrument calibration were observed challenge during the study paired specially for Genxpert instrument.

7. Strengths and limitation of the study

The study provides information about the performance of Xpert MTB/RIF assay, MGIT culture. Moreover it informs on how Xpert MTB/RIF assay& MGIT culture tests perform at the point of treatment, where the assay and test can have greatest impact on patient care in remote health care facility. However, this study is not without limitations. The following were the limitation of the study.

- Due to the nature of the study design (cross-sectional), we are unable to clarify culture negative Xpert MTB/RIF positive samples, whether it is false positive or not, using follow up culture and clinical data.
- Operational and logistic difficulties to link, Culture result with clinical outcomes of the study participant.

8. Conclusion and Recommendation

9.1 Conclusion

Our findings demonstrate that both Xpert MTB/RIF assay and MGIT is a useful tool for the detection of MTBC with high sensitivity in extra pulmonary tuberculosis suspected specimens compared with conventional AFB smear microscope. Negative Xpert MTB/RIF result might be insufficient to rule out active TB

The burden of Genxpert and Culture confirmed extra pulmonary tuberculosis infection was low. The most prevalent type of extrapulmonarytuberculsis was gastric lavage tuberculosis followed by TB from Peritoneal fluid. There was a significant difference between GeneXpert results compared with Culture results. A significant proportion of EPTB cases were also co-infected with HIV.A more accurate test could contribute EPTB case detection, and thus reducing the morbidity and mortality

9.2 Recommendation

Early diagnosis of TB should be strengthened on children families, caregivers and household contacts with clinical and treatment history

Positive Genxpert, but Culture negative results should be read cautiously and be well correlated with clinical and treatment history of the patients

Further studies and justifications required to clarify operational difficulties, challenges, and limitations on Gen Xpert MTB/RIF and MGIT in current TB control/treatment in the country.

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10. ANNEXES

11.1 Annex1: Information sheet for participants family

Date ----------

Addis Ababa University, Department of Medical Laboratory Science, Collage of Allied Health Sciences,, Addis Ababa, Ethiopia.

Hello my name is **AzmachBiset**. Currently am studying in Addis Ababa University, department of medical laboratory science and undertaking a master's degree (Msc) in Diagnostic and Public health microbiology on determining the prevalence of TB from body fluid samples from presumptive EPTB by gene xpert machine and compare the findings with Culture (MGIT/LJ).
First of all I would like to thank you in advance for your valuable cooperation and participation in this study with fully completing the consent form. Could you please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely without any hesitation**.**

Title: Prevalence of EPTB Children and riff resistance by gene xpert assay from body fluid samples in in St.Luke catholic hospital,Atat Hospital,Ameya Hospital woliso and Dilela HCs and TulluBollo Hospital.

Background: Tuberculosis is an infectious disease caused by various strains of mycobacteria, usually *Mycobacterium Tuberculosis* the diagnosis of EPTB is usually established by examination of hispopathological samples, and ZN stained smears and culture. However, rapid and sensitive diagnostic tool should be used since the above mentioned diagnostic tests are not sensitive, time consuming and ref resistance detection is not possible. In order to overcome this problem other more sensitive diagnostic instrument who replaces the conventional method should be addressed. Since tubercle bacilli or their nucleic acids are also expected to be found in body fluid samples, it was interested to assess all body fluids for the case of diagnosing E PTB.

Objective of the study: The aim of this study is determining and detecting of EPTB by gene xpert machine and compares the findings with MGIT/LJ culture in St.Luke catholic hospital,Atat Hospital,Ameya Hospital woliso and Dilela HCs and TulluBollo Hospital, besides EPHI TB laboratory, Ethiopia.

Participation: The process and procedure was carried out after I get your valuable willingness to be engaged /or allow your child to participate. All volunteer Children/patients with EPTB is understand this study and allow them to participate who fulfilling inclusion criteria was included.

Benefit of the study:your child was benefited from the study; because it was part of your and/or child diagnosis and might be a key to your child current and future problem if it will come up/end up with positive result.

Compensation: You will get your child result through the physician who requested the test. Your child will get treatment for free if he/she becomes positive for EPTB.

Risks and complication

There will not be any anticipated risks to your child participation. As routine laboratory procedure body fluid sample was taken once from yourchild from different organ. During sample collection your child may feel some discomfort but this will not produce any serious problem.

Confidentiality of the study

There was no any sensitive issue that your child was asked with regard to your social desirability but any information that is obtained in connection with this study and that may be identified with your child will remain confidential. Participants will not be prohibited to stop or withdraw at any time from the study. Only interested participants can retrieve their own lab result using their code number. The information collected about your child was coded using numbers. No personal information was disclosed to third party or will not appear in any report from this study except your physician.

Approval: This research project has got ethical clearance from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences School of Allied Health Sciences, Department of Medical Laboratory Science.

Whom to contact: If you have any question or description about this study, you can communicate on the following address:

1. Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences

Tel: +251-112-75-51-70

Fax: +251-112-75-46-69

E-mail: SMLT@ethionet.et

P.o.Box: 1176, Addis Ababa, Ethiopia

2. Principal Investigator: Azmach Biset (Bsc)

Tel. +251-911753745

E-mail: azmach2004@gmail.com

11.2 Annex II: Assent Form for children aged 12-15 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 (Peritoneal fluid, Pleural fluid, Pus, CSF, Synoveal, Ascitic Lymphnode, Gastric aspirate)

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, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy) _____

If Parents /families are illiterate;

Name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy) _____

Phone number (parents/guardians)_____

Name of Principal investigator, date and signature of Principal investigator

_____ _____/____/_____ (dd/mm/yy) _____

11.3 Annex III: Informed consent form

Dear sir /Madam

Hello my name is AzmachBiset; currently I am studying in Addis Ababa University, department of medical laboratory science and undertaking a master's degree (Msc) in Diagnostic and Public health microbiology on determining the prevalence of TB from body fluid samples from presumptive EPTB by gene xpert machine and compare the findings with MGIT/CULTURE

I like to ask all presumptive EPTB patients/guardians of the patients in case of children requested for gene xpert to participate in responding the questioners.

Signature of person taking the consent/assent_____

Date _____D/M/Y

I read the information or have been read to me. I have had the opportunity to answer the question on the questioner. I consent/assent voluntary give permission for my child sample to be used in the current research project: determining the prevalence of TB from body fluid samples from presumptive EPTB by gene xpert machine and compare the findings with MGIT/CULTURE

Name of participant/_____signature_____

Date _____ D/M/Y

Data collector name _____signature_____

Date _____ D/

11.4 Annex IV: Data collection sheet (English Version)

Section I

Data collection sheet

I .patients identification

1. Code No.: _____
2. Sample ID: _____
3. Date: _____

4. Age _____ (years)

5. Sex

- Male
- Female

6. Recidence

- Rural
- Urban

7. Educational status

- primary
- No formal education

II.TB disease and treatment history

1. Site: extra pulmonary (specify).____
2. Registration group:

- New
- Relapse
- MDRTB Contact

3. Request for testing

- Presumptive TB
- presumptive MDRTB

4. Co-infections

- yes (specify)_____
- No

5 .specimen appearance

- Clear
- turbid
- bloody
- Muccoid

6. Data collector: Name_____

Date_____

Signature_____

11.5 Annex V: Data collection sheet (Amharic Version)

ስሜ ዘማቹ በሰ ጥይባላል፡ ፡ በአሁኑሥዓትበአ.አ.ዩ.የ ጤና ሳይንስኮሌጅትምህርትክፍልበህክምና ላቦራቶሪሁለተኛዲግሪዬን በስነ ረቂቅተህዋስያትምርምርእና በህብረተሰብጤና (DiagnosticandPublicMicrobiology)ላይGeneExpertበሚ ባልመሳሪያከሳንባወጪ ሆነ ቲበበሽታላይምርመራእያካሄድኩእገ ኛለሁ ፡ ስለዚህከሳንባወጪ ሆነ ቲቢያ ላቦራቶሪምርመራ ለማድረግ/ለማግኘትየ ምትማጡቃካሜዎችበፈቃደኝነ ትላይየ ተመሰረተየ ቃል/የ እፍጥያ ቄእንድትሞሉልኝበአክብሮትእጠይቃለሁ ፡ ፡

የ ተሳታፊስም--------------------------------- ፊርማ-----------------------

ቀን -------------------------------

ይህንንስምምነ ትአን ብቤ/ተነ ቦልኝተረድቻለሁ ፡ እኔ ምይህንንእደልበማግኘቴጥያ ቄዎችንለመመለስፈቃደኛበመሆኔ ከሳንበ ሰወጪ ሆነ የ ቲበብሽጳእና ሜድሐኒ ትየ ተላመደቲኒ በሽታሐከምስ ሜጀ ዝልኝምርመራፈቃደኛመሆኔ ንአረጋግጣለሁ ፡

የ ተሳታፊስም--------------------------------- ፊርማ-----------------------

ቀን ----------------------------

11.6 Annex VI:Data collection sheet (AfanOromiffa Version)

MaqaankooAzmacheeBiseetee.Ammaan kana YuunivasiitiiFinfinneettiMuummeeYaalaFayyaaNamaa,dippaartimentiilaaboraatooriifayyaanam aattidigriilammaffaawal'ansaa fi fayyaanamaalubbuqabeeyyiiijaanhinmul'anne (Diagnostic and Public health microbiology) irrattiqorannoodhukkuba TB sombaanalaairrattimeeshaa gene xpertjedhamuungaggeessaanjira.KanaafuunamootnidhukkubaTB sombaanalaatiifyaaliilaboratooriiargachuufdhuftanyoofedhiiqabaattangaafiiafaaniiakkanaafguutt ankabajaananisingaafadha.

Maqaanamahirmaatee_____ mallattoo_____

Guyyaa_____

Waliigaltee kana dubbiseeraykknaafdubbifameera.Aniscarraa kana argadheeakkandeebisuuneeyyamamaata'eeqorannooogeessifayyaayaalakootiifajajeirrattiyaalii TB sombaanalaa fi qorichawajjinwal bare kanjedhuirrattiakkagaggeessatanfedhiikoota'uumirkaneesseera.

Maqaahirmaataa _____ mallattoo_____

Guyyaa _____

11.7 Annex VII: Gene Xpert MTB/RIF (Laboratory data)

				Genxpert Result			
				MTB ND	MTB D& RIF ND	MTB D& RIF INT	MTB D& RIF R
No.	Sample ID	Sample collection date	Sample processing date	MTB not detected	MTB detected & RIF not detected	MTB Detected & RIF Indeterminate	MTB Detected & RIF Resistant

.

11.8 Annex VIII Culture result collection sheet (Laboratory data)

No.	SampleID	Sample collection Date	Sample processing date	LJ result	MGIT 960 result	DST result	Culture result date	DST result date	Remark

11.9 Annex IX : Standard Operating Procedure for Gene xpert assay

10.8.1 Principles and Procedures of Xpert MTB/RIF assay

Test principle:

The Xpert MTB/RIF assay consists of a single use multichambered plastic cartridge preloaded with the liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction, and heminested real-time PCR. The PCR assay amplifies a 192 bp segment of the M. tuberculosisrpoB gene in a heminested real-time PCR. The internal control heminested B.globigii assay is multiplexed with the M. tuberculosis assay. M. tuberculosis is detected using five overlapping molecular beacon probes (probes A to E) that are complementary to the entire 81-bp RIF resistance determining core region of the wild type rpoB gene. Mutations in the rpoB gene target inhibit hybridization of one or more of the rpoB specific molecular beacons, reducing or eliminating the signal from the corresponding probes.

Sample preparation:

The extra pulmonary samples are treated with sample reagent (SR). The sample reagent contains NaOH and isopropanol. The SR is added at a 2:1 ratio to the sputum sample and incubated for 15 min at room temperature. The treated sample is transferred into the cartridge, the cartridge is loaded into the GeneXpert instrument, and an automatic process completes the remaining assay steps.

Assay cartridge:

The assay cartridge contains:

- Lyophilized Bacillus globigii spores which serve as an internal sample processing and PCR control
- Processing chambers
- Valve body
- Reaction tube

Running a test:

Each GeneXpertDx module processes one sample. insert the sample and applicable reagents into a GeneXpert cartridge, create a test, load the cartridge into an available instrument module, and then start the test. During the test, the system performs the following steps:

- Moves the sample and reagents into different chambers in the cartridge for sample

preparation. 40

 Hydrates the reagent beads

 Performs probe checks to ensure that the sample preparation is successful (only if the assay definition requires this step).

 Moves the sample and reagent mixture into the reaction tube.

 Starts the PCR cycles and real-time detection

System Calibration:

The thermal reaction chamber thermistors are calibrated to ± 1.0 °C using National Institute of Standards and Technology (NIST)-traceable standards. During the manufacturing process, the temperature of the heating system is measured at two temperatures: 60 °C and 95 °C. Calibration coefficients that correct for small errors in the raw thermistor readings of the heaters are stored in the memory of each I-CORE module. The optical system is calibrated using standard concentrations of individual unquenched fluorescent dye-oligos. For each optical channel, the signal produced by a tube alone (the blank signal) is subtracted from the raw signal produced by the dye-oligo standard to determine the spectral characteristics. Using the individual spectral characteristics of the pure dye-oligos, signals from an unknown mixture of dye-oligos can be resolved into corrected signals for the individual dye-oligos in the mixture.

10.9 Standard Operating Procedures (SOPs) for TB culture

Specimen Digestion/Decontamination for Mycobacteriology Culture

Analytic Procedure

Specimen types

 Specimens that require decontamination include Peritoneal fluids,CSF,Pus,Pleural fluids, Lymphnode,Synoveal fluid, Ascitic fluids, , bronchial secretions, washings, or biopsies, skin, soft tissue, gastric lavage, stool specimens, urines and all other specimens from sites contaminated with normal microbial flora.

 When a specimen is determined to be unacceptable, a repeat specimen must be requested.

 Unacceptable specimens include unlabeled or inadequately identified specimens, dry swabs,

and those received in previously used containers, containers that are non-sterile or cannot be tightly sealed.

 Specimens held unrefrigerated prior to processing for greater than one hour may lead to

bacterial overgrowth at levels that prevent detection of mycobacteria

 Sputum specimens collected at different times must never be pooled as this greatly increases

the contamination rates.

Specimen storage

 Specimens should be delivered to the laboratory as soon as possible to avoid overgrowth by

contaminants and normal respiratory flora.

 Specimens not processed within one hour of collection must be refrigerated at 2 – 8° C.

Reagents/Media:

Reagents used for digestion/decontamination are dependent on precise adherence to the required

procedures.

1. Fresh working NALC-NaOH Solution - Directions for preparing NALC-NaOH Solution or alternatively BD BBL™ MycoPrep™ Specimen Digestion/Decontamination Kit are included

2. Phosphate buffer solution (pH 6.8)

3. MIT™ Mycobacteria Growth Indicator Tube

4. BACTEC™ MGIT™ 960 Supplement Kit including:

a. BACTEC MGIT Growth Supplement 42

b. MGIT PANTA Antibiotic Mixture vial

Required Supplies:

1. 50 ml sterile conical centrifuge tubes

2. Vortex mixer

3. Centrifuge- capable of speed 3,000–3,500 x g, fixed angle rotor with aerosol-free safety centrifuge cups

4. Funnel and waste container filled 1/3 full with an approved disinfectant solution

5. Disposable sterile pipettes

6. Slide warmer set between 65 and 75°C

7. Glass microscope slides (a frosted end for labeling with a pencil is useful)

Quality Control:

Negative - Process a negative water or buffer control with each run of specimens.

Positive - Sputum spiked with a mycobacterial species should be processed as a positive control once per week.

Digestion, Decontamination and Concentration Procedure Steps:

Follow laboratory bio-safety practices for all procedure steps.

1. Prepare fresh working digestant/decontamination solution or BD MycoPrep™ Specimen Digestion/Decontamination Kit.

2. Prepare the BSC for use following the Use of the Bio-Safety Hood in the Mycobacteriology Laboratory SOP.

3. Reconstitute a lyophilized vial of MGIT PANTA Antibiotic Mixture with 15 mL of

BACTEC MGIT Growth Supplement. *Once reconstituted, the PANTA mixture must be stored at 2 – 8°C and used within 5 days.

4. Label the MGIT tubes with the specimen number.

5. Unscrew the cap and aseptically add 0.8 mL of Growth Supplement/MGIT PANTA

Antibiotic Mixture to each labeled MGIT tube. *For best results, the addition of Growth Supplement/MGIT PANTA Antibiotic Mixture should be made just prior to specimen inoculation. 43

6. If the specimen was not collected in a sterile, labeled 50 ml disposable centrifuge tube, transfer the entire specimen to a labeled 50 ml conical tube. *No more than 10 ml of specimen may be processed per conical tube. The remaining sample should be transferred to second tube and processed. Repeat for all patient specimens.

7. Stagger the tubes in the rack to prevent cross contamination. *Do not process more specimens in a batch than the centrifuge will hold.

8. Opening only one tube at a time, to the first specimen tube add an equal amount of fresh

working NACL-NaOH solution, rotate and invert the tube, ensuring the mixture coats the

entire interior surface. Vortex the mixture for 10-15 seconds. Repeat this step for each

specimen in the batch.

*Start the timer for 15 minutes after adding the solution to the first tube in the batch.

9. Allow the specimens to stand the entire 15 minutes. During the incubation time check each

specimen by slightly tilting the tube and observing for liquefaction.

*If a specimen is very mucoid with no change during these checks, add a small amount of NALC directly to the tube, vortex and allow to stand until the end of this incubation time.

10. After the digesting has remained in the first tube for 15 minutes, begin with the first specimen and fill the first tube to the 50 ml mark with phosphate buffer by slowly pouring the buffer down the side of the tube avoiding splashing or contamination. Tighten the cap and

wipe the outside of each tube with the disinfectant soaked towel, then invert the tube several times to mix thoroughly. Repeat this step on each of the remaining specimens in the batch, mixing well after each addition.

11. Load tubes in aerosol-free safety centrifuge cups. Centrifuge tubes for 15 minutes at 3,000 -

3,500 x g. Allow aerosols to settle a few minutes before removing tubes from the centrifuge cups.

12. Opening one tube at a time, pour off the supernatant into a waste container filled 1/3 full with

approved disinfectant solution. Wipe the lip of the conical tube with a disinfectant soaked

towel and then recap.

*The use of a funnel is preferred. (Pour slowly so as not to disturb the pellet and be sure to

not touch the funnel while pouring. 44

13. Using a sterile disposable transfer pipette, re-suspend the pellet by adding 1-2 mL of

phosphate buffer. Gently mix the tube contents.

14. Add 0.5 mL of the concentrated specimen suspension to the prepared BBL™ MGIT™

Mycobacteria Growth Indicator Tube. Also add a drop (0.1 - .25 mL) of specimen to a

Lowenstein-Jensen agar slant or other conventional solid medium.

*Refer to the Mycobacteriology Culture SOP.

15. With the same pipette, place a drop of the suspension onto a clean, labeled glass microscope slide. Prepare a smear over an area of 1 by 2 cm of the slide.

*Refer to the AFB smear SOP.

16. Tightly recap the MGIT tube and mix well. Leave the inoculated tubes at room temperature for 30 minutes before loading in to the MGIT system.

*Refer to the Mycobacteriology Culture SOP.

17. Load inoculated BBL™ MGIT™ Mycobacteria Growth Indicator Tube into the instrument following manufacturers' instructions for the duration of the recommended 42 day testing protocol. 18. For specimens in which mycobacteria with different incubation requirements are suspected, a duplicate MGIT tube can be set up and incubated at the appropriate temperature; e.g., 30 or 42°C. Inoculate and incubate at the required temperature. These tubes must be manually read (refer to the BACTEC MGIT Instrument User's Manual).

Post-analytic Procedure

Specimen Retention:

Store processed specimens in AFB refrigerator for as long as space allows (approximately two weeks).

Calculations:

Quality monitors are compiled monthly to monitor the digestion process of the lab. 45

Expected Values:

 Contamination rates of 8 - 10% are considered to be acceptable for solid culture media.

 Liquid culture media will have a higher contamination rate which was monitored for acceptability.

 A contamination rate of less than 5% suggests overly harsh decontamination.

 A contamination rate of greater than 10% growth suggests inadequate decontamination, incomplete digestion, reagent and/or media contamination or environmental contamination.

Interpretation of Results:

Contamination rates higher than 10% on solid media was investigated to determine whether equipment, reagent or personnel are causing the high rates. Data is included in the monthly quality assurance report.

Method Limitations:

 The procedure is dependent on strict adherence to recommended techniques, timing, temperature, and biochemical requirements. Any deviation from the SOP will not provide appropriate clinical care.

 The NaOH procedure is very robust and may kill up to 60% of tubercle bacilli in clinical

Specimens, and may give a false negative result, especially in cases of paucibacillary disease

as seen in early disease, or in many HIV positive patients.

 Additional contributory factors such as heat build-up in the centrifuge step may also kill

tubercle bacilli.

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: Azmach Biset /BSc/

Signature: ----------

Date of submission: ----------

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

Name: Kassu Desta /BSc, MSc, PhD scholar /

Signature: ----------

Date of submission: ----------