

**DIAGNOSTIC METHODS USED FOR PEDIATRIC TB IN SUB-SAHARAN AFRICA: A
LITERATURE REVIEW**

by

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ABSTRACT

The purpose of this review is to investigate the gap in pediatric TB diagnosis and reporting, and identify interventions that have been found to be effective in Sub-Saharan Africa. In 2017, one million children became infected with TB and 230,000 of these children died. With early diagnosis, child mortality resulting from TB infection can be prevented. TB manifests differently in children; thus, most conventional diagnostic tools fail. In Sub-Saharan Africa alone, it is estimated that 20% of all active TB cases are in children. Since childhood TB is a major public health issue, there is a dire need for better diagnostics and reporting systems. Moreover, TB is the leading cause of death in HIV positive individuals making it important to discuss HIV status and other comorbidities affecting diagnosis and reporting of pediatric TB. BCG vaccination can also interfere with TB diagnosis and high BCG vaccination rates make accurate diagnosis difficult due to cross reactivity with some diagnostic methods. Many children in high TB endemic areas like Sub-Saharan Africa suffer from severe acute malnutrition which complicates early diagnosis in children. For the purpose of this review, HIV positive individuals and malnutrition status will be included in the analysis as well as BCG vaccination rates because all pose challenges to diagnosing childhood TB in Sub-Saharan Africa.

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LIST OF ACRONYMS

Mtb	<i>Mycobacterium tuberculosis</i>
TB	Tuberculosis
AM	alveolar macrophage
LTBI	latent Tuberculosis infection
TST	tuberculin skin test
IGRA	interferon gamma release assay
PPD	purified protein derivative
NAAT	nucleic acid amplification test
RIF	Rifampicin
ELISA	Enzyme linked immunosorbent assay
ELISpot	Enzyme linked immunosorbent assay
BCG	Bacillus Calmette Guerin
HIV	Human Immunodeficiency Virus
SAM	severe acute malnutrition
PPD	purified protein derivative
NAAT	nucleic acid amplification test
MDRTB	Multi-drug resistant Tuberculosis
XDRTB	extensively drug resistant tuberculosis
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
UNICEF	United Nations International Children's Emergency Fund
UNDP	United Nations Development Program
CT	cycle threshold
SFU	spot forming units
MGIT	mycobacteria growth indicator tube
TTP	time to positivity
IS	induced sputum

GLA	gastric lavage aspirate
NPA	nasopharyngeal aspirate
CSF	cerebral spinal fluid
LAM	lipoarabinomannan
FNA	fine needle aspirate
EPTB	extrapulmonary tuberculosis
AFB	acid fast bacilli
IFNg	interferon gamma

PREFACE

I would like to thank my family and friends, but most importantly my mother and my sister, for the never-ending love and support they gave me throughout the entirety of my educational journey. I want to give a special thanks to my essay advisor Dr. Joshua Mattila and essay reader Joanne Russell for the guidance and encouragement during my graduate school education.

Finally, I would like to thank the University of Pittsburgh for providing me with an extraordinary education and inspiring me to pursue a career in the field of public health.

1.0 INTRODUCTION

1.0 MTB PATHOGENESIS

Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis* (Mtb). Mycobacteria are slow growing acid-fast bacilli that live inside phagocytes including macrophages and have a high concentration of mycolic acids found in the bacterial cell wall (Glickman et al., 2001). This characteristic is the basis of some diagnostic tools used to detect presence of the bacterium. In addition to the presence of mycolic acids, the cell wall also contains waxy components which serve as another virulence factor (Glickman et al., 2001). Mycobacteria have protein secretion systems contribute to their virulence. ESX1-ESX5 play integral roles in the secretion of proteins that disrupt host immune responses (Glickman et al., 2001). ESX1, encoded by the genomic RD1 region, secretes the proteins ESAT-6 and CFP-10 that are required for bacterial intracellular bacterial survival (Glickman et al., 2001). The secretion of these proteins is the basis for some diagnostics that detect the absence or presence of the antigens that these proteins secrete.

Mtb infection presents differently in children and this poses challenges for diagnostics and detection. Granulomas, the lesions associated with TB, are paucibacillary in children, meaning there is a small bacterial load making it more difficult for diagnostics to detect disease causing positive cases to appear falsely negative (Piccini et al., 2014). Signs and symptoms, to be discussed in further detail, often overlap with other diseases experienced in children of high TB burden areas making diagnosis more complicated (Orikiriza et al., 2018).

1.1 HOST RESPONSE TO MTB

TB is transmitted via aerosols. Tubercle bacilli can be inhaled by the host through close proximity contact with an infected person by activities including talking, coughing, or sneezing (Fennelly et al., 2004). Once bacteria enter the host, Mtb are phagocytosed by alveolar macrophages (AM) in the alveoli of the lungs (Glickman et al., 2001). Mtb have evolved to survive inside of macrophages and will begin to replicate within the AM, eventually spreading to other cells nearby (Glickman et al., 2001). With the help of lymphatics, cells can reach parts of the body separate from pulmonary tissue and cause extra pulmonary disease (Glickman et al., 2001). The interaction between the host immune system and the mycobacteria drive formation of granulomas. When the host's adaptive immune response is initiated, granulomas play important roles by controlling bacterial growth and restricting it within a confined area, but granulomas also provide a site for successful growth of the bacteria (Glickman et al., 2001).

The immune response to TB infection in children is different than what is experienced in adults. It is estimated that it takes 4-12 weeks from time of infection for a child's adaptive immune response to recognize TB infection (Thomas, 2017). Infants and children have downregulated innate cells that ultimately impact the progression to severe active disease (Marais et al., 2004). Children are more likely to develop severe forms of disease because the innate immune system cannot properly tame infection resulting in lymphatic spread to other parts of the body (Marais et al., 2004). The most common types of extrapulmonary disease experienced in children are TB lymphadenitis and TB meningitis (Piccini et al., 2014). TB lymphadenitis is the inflammation of the lymph node and develops around 6 months after infection (Piccini et al., 2014). TB meningitis is when infection spreads to the central nervous system and is more common in younger children (Piccini et al., 2014).

1.1.1 ACTIVE TB INFECTION

Upon infection with Mtb, it can either result in an active or latent state. Active TB is infectious and can be spread to other people through aerosols (Centers for Disease Control and Prevention [CDC], 2016). Active TB infection should appear positive on all diagnostic exams (CDC, 2016). Clinical manifestations are site specific and correspond to the area of infection (CDC, 2016). Pulmonary TB symptoms may include but are not limited to coughing, fever, weight loss, and night sweats (Cruz et al., 2007). Extrapulmonary disease may have more serious signs and symptoms including adenopathy, tuberculomas, and spondylitis (Cruz et al., 2007). Other symptoms exist when infection spreads to other parts of the body. Drug susceptible active TB disease could be treated using first line agents Isoniazid, Rifampin, Ethambutol, or Pyrazinamide with durations ranging from 6 to 9 months.

1.1.2 LATENT TB INFECTION

Latent TB is characterized by the lack of clinical evidence and negative diagnostic results. In latent TB, disease cannot be transmitted to other individuals (CDC, 2016). The Tuberculin Skin Test (TST) and Interferon Gamma Release Assays (IGRA) can detect latent disease but cannot differentiate between the active and latent state (CDC, 2016). If latent disease is detected, proper treatment should be started immediately due to the possibility of reactivation to an active state. Treatment should be modified to fit the patients' needs taking into consideration immunocompetency and contact with a drug resistant infected individual. Latent TB can be treated using Isoniazid, Rifapentin, or Rifampin (CDC, 2016). The lifetime risk of reactivation of someone with latent disease is 10% (CDC, 2016).

1.1.3 HIV INFECTION EXACERBATES TB

HIV-associated TB is highest in the WHO classified African region (World Health Organization [WHO], 2017). Eighty two percent of TB patients also are positive for HIV (WHO, 2017). In 2017, there were .9 million new cases of TB among HIV-positive individuals with 72% living in Africa alone (WHO, 2017). Further, TB is the leading cause of death for HIV-positive people (WHO, 2017). In 2016, 40% of HIV deaths were attributable to TB infection (WHO, 2017). HIV positive people are 20-30 times more likely to become infected with active TB (WHO, 2017).

Figure 1 shows the estimated HIV prevalence in new and relapsing TB cases by country in 2016 (WHO, 2016).

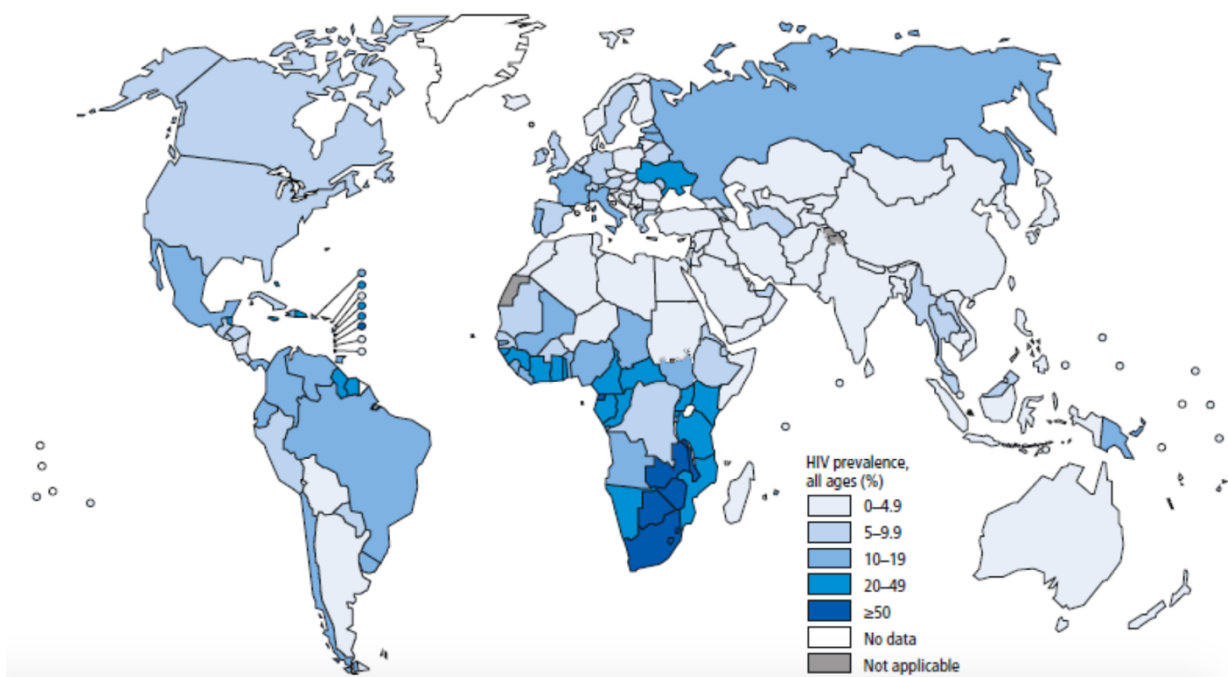


Figure 1. World map of estimated HIV prevalence in new and recurring TB cases in 2016

1.1.4 BCG VACCINE

The Bacille Calmette-Guerin (BCG) vaccine is made from an attenuated strain of *Mycobacterium bovis* and is the only vaccine available for the prevention of TB (Luca et al., 2013). BCG vaccination should only be used in people who have not been infected with Mtb and are at high risk of exposure to TB (Cernuschi et al., 2018). This vaccine is effective at preventing childhood TB but is not as effective for adult prevention (Glickman et al., 2001). It is estimated that BCG has a 60-80% vaccine efficacy for children with more severe forms of infection including TB meningitis (Roy et al., 2014). It is recommended that a TST be done prior to verify the child is not infected with TB (Food and Drug Administration [FDA]). In endemic countries, it is also recommended that an HIV test be done prior to receiving vaccination (WHO, 2018). Almost all of Africa, including sub-Saharan Africa, currently has a national BCG vaccination policy for all (Mihigo et al., 2017). **Figure 3** shows which countries have a national BCG vaccination policy (BCG World Atlas, 2017).

1.1.5 MULTI DRUG RESISTANT TB

Multidrug-resistant TB is defined as a strain of Mtb that is resistant to first line antibiotic treatment using Isoniazid and Rifampicin (WHO). Globally, it is estimated that 490,000 people developed MDRTB in 2016 (WHO, 2016). Of the estimated incidence of MDRTB cases in 2016, only 22% of cases began treatment (WHO, 2016). Extensively drug-resistant TB (XDRTB) is a form of MDRTB but has further resistance to second line anti-TB drugs including fluoroquinolones and at least one of the following injectable second line drugs Amikacin, Capreomycin, Kanamycin, Streptomycin, Ethionamide, Cycloserine, Linezolid, or Clofazimine

(WHO). Of the estimated MDRTB cases in 2016, 6.2% were estimated to also be XDRTB (WHO, 2016). There is not much data on MDRTB and XDRTB in children because diagnosis is difficult leading to underreporting and underdetection. In a mathematical modelling study, it was estimated that of the 279,824 child TB cases in the African region, 4,736 of those cases are MDRTB (Jenkins et al., 2014).

1.2 TB DIAGNOSTICS

1.2.1 TUBERCULIN SKIN TEST/ PURIFIED PROTEIN DERIVATIVE SKIN TEST

The Mantoux tuberculin skin test (TST) is an intradermal injection of 5 Tuberculin Units of purified mycobacterial antigens into the inner surface of the forearm (Huebner et al., 1993). Forty-eight to 72 hours after the initial injection, the area of injection needs to be observed by a trained health professional and is evaluated using a classification system which measures the induration in millimeters (CDC, 2011). If the patient has been exposed to mycobacteria and has generated adaptive immunity against mycobacterial antigens, injection will lead to a delayed-type hypersensitivity response (Huebner et al., 1993). There are different classifications based on the background of the patient. The observed millimeter induration decreases as the risk for TB infection increases, for example, an HIV positive individual is classified as Mtb positive if the purified protein derivative (PPD) injection leads to an induration of 5 or more millimeters while for a person with no known risk factors, an induration of 15 or more millimeters is considered positive (CDC, 2011). PPD testing is inexpensive, making it an affordable option in low income countries. The injection is easy to administer as well as read. Although, it does require more than

one visit and lacks specificity in BCG vaccinated individuals which is a problem in countries with high BCG vaccination rates.

1.2.2 INTERFERON GAMMA RELEASE ASSAYS

Interferon Gamma Release Assays (IGRAs) are blood tests that measure IFN-gamma production by antigen-specific T cells in response to ESAT-6 and CFP-10 proteins, two antigens that are not present in the BCG vaccine or other mycobacteria (Pai et al., 2008). The measurement of IFN-gamma can be done by ELISA or ELISpot tests (QuantiFERON-TB Gold In-Tube and T-SPOT TB, respectively). IGRAs can detect both latent and active Mtb infection but cannot differentiate these states. When using an ELISA-based test, a patient is characterized as positive for Mtb if the IFN-gamma concentration is greater than or equal to 0.35 IU/ml (Qiagen Group, 2017). When using the ELISpot test, a positive result is reported when the number of IFN-gamma producing cells (SFU) is greater than eight in the TB antigen wells (Oxford Immunotec, 2018). IGRA testing is more expensive than PPD testing and requires specialized equipment in an appropriate laboratory environment. The assay is not as simple to read as PPD testing and requires more training of laboratory personnel.

1.2.3 MICROBIOLOGIC DIAGNOSIS

Acid fast staining can be done on sputum or other specimens from the patient including cerebrospinal fluid, blood, pleural effusions, or tissue from a suspected TB case. Ziehl-Neelson staining uses multiple reagents including primary stain Carbol Fuchsin, decolorizer acid-alcohol, and counterstain methylene blue which will differentiate between non-acid fast bacilli and acid fast bacilli (Hussey & Zayaitz, 2018). Acid fast bacilli will retain the primary color red and non-

acid fast bacilli will wash clear after decolorization and retain the counterstain blue color. The presence of acid-fast bacilli are indicative of Mtb infection (Hussey & Zayaitz, 2018). Another popular stain to detect Mtb is auramine-rhodamine. Auramine-rhodamine staining uses fluorescence microscopy as a readout. This stain uses Auramine Rhodamine solution as the primary stain, acid alcohol as the decolorizer and potassium permanganate as the counter stain (Hussey & Zayaitz, 2018). Acid fast samples will retain the primary stain and appear red/orange against the dark background and non-acid fast samples will not fluoresce against the dark background or might appear a very light yellow (Hussey & Zayaitz, 2018). Microbiologic diagnosis requires a specialized laboratory environment and properly trained laboratory personnel.

1.2.4 NUCLEIC ACID AMPLIFICATION TEST

The most common NAAT used is the Xpert MTB/RIF (Cepheid, 2015). Xpert simultaneously tests patient samples for TB and Rifampicin resistance. GeneXpert is a qualitative, real time PCR diagnostic test that uses probes to identify gene segments specific to Mtb. The primers used amplify portions of the *rpoB* gene and differentiate between mutated and nonmutated sequences. Rifampicin resistance is caused by a mutated *rpoB* gene. Rifampicin targets the products of the Mtb *rpoB* gene and when it is mutated, Rifampicin can no longer bind the RNA polymerase making it lose its antimicrobial properties (Telenti et al., 1993). Probe thresholds are set and used for MTB/RIF analysis. Mtb is detected if it has a total cycle threshold (CT) value of less than 2 (Cepheid, 2015). RIF resistance is detected if the CT is greater than 4.0 (Cepheid, 2015). RIF resistance is not detected when less than 4.0 (Cepheid, 2015). Mtb is not detected when there is none or only one positive probe found (Cepheid, 2015). For easy interpretation, a lab technician

does not have to calculate the test results on their own and instead, the instrument will indicate whether Mtb and RIF is detected. The turnaround time is less than 2 hours allowing for diagnosis and treatment to be done in one initial visit. Xpert only detects resistance to Rifampicin and does not perform full drug susceptibility testing. Xpert technology is more expensive and requires a specialized laboratory environment in addition to reliable electricity.

1.3 GLOBAL TB BURDEN

Mtb ranks number nine for the leading cause of death worldwide. It is the number one cause of death by a single infectious agent (WHO, 2017). Although there has been progress made in decreasing mortality and incidence rate, it still poses as a serious public health issue. In 2016, 10.4 million people suffered from TB infection (WHO, 2017). This number is disproportionate among different demographics. Ninety percent were adults, 65% were male, 10% were living with HIV (with 75% of cases in Africa alone), and 56% of all cases were in five countries: India, Indonesia, China, the Philippines and Pakistan (WHO, 2017). Of the cases notified in 2016, 6.9% were estimated to be children under age 15 (WHO, 2017).

1.3.1 SUB-SAHARAN AFRICA

It is estimated that by 2030, the number of children under 18 in Sub-Saharan Africa will increase from 496 million in 2015 to 661 million (United Nations International Children's Emergency Fund [UNICEF],2015). Forty five percent of the population in Sub-Saharan Africa is under the age of 15 (UNICEF,2015). The increasing number of young people also increases the risk of acquiring TB infection. Infectious diseases like TB can partially explain the typical pyramid

shape of Sub-Saharan Africa's population. **Figure 2** shows the population pyramid of Sub-Saharan Africa. The health profile of Sub-Saharan Africa impacts the overall burden of TB experienced in children and proves as a serious challenge in diagnosis and reporting.

Sub-Saharan Africa ▼ 2017

Population: 1,014,318,745

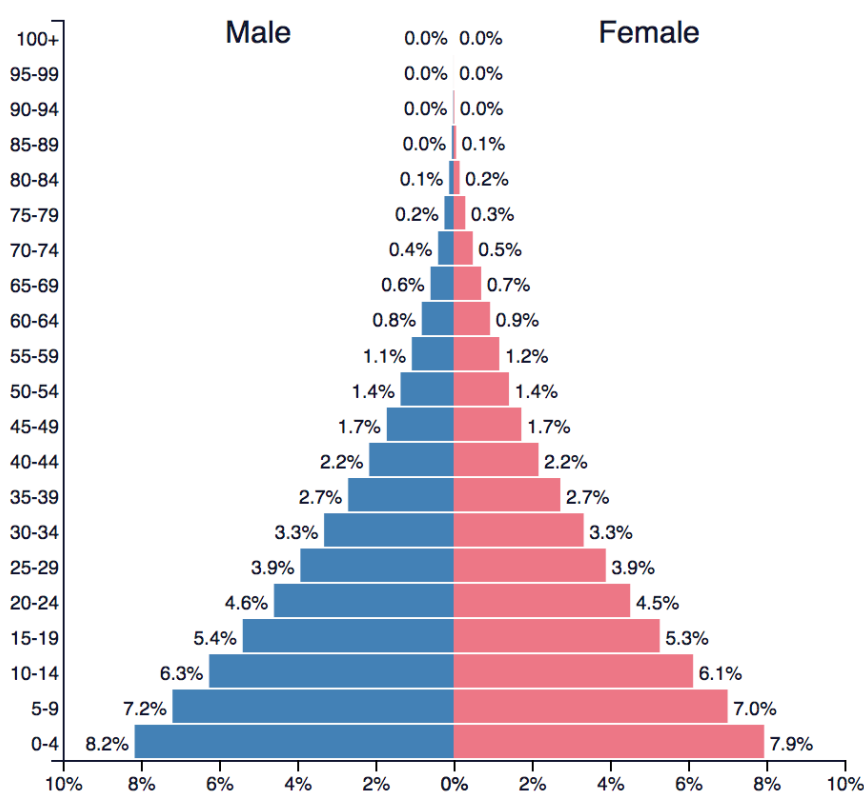


Figure 2. 2017 Population pyramid for Sub-Saharan Africa

Sub-Saharan Africa alone, accounts for greater than 70% of the world's HIV infection (Kharsany et al., 2016). Of the 1.5 million AIDS related deaths in 2013, Sub-Saharan Africa accounted for 74% of that total (Kharsany et al., 2016). Ninety percent of children in the world

suffering from HIV infection also live in Sub-Saharan Africa (WHO, 2015b). Seventy-nine percent of HIV/TB coinfections are in Sub-Saharan Africa (Lawn et al., 2009). Since HIV weakens the immune system, it increases the risk for active TB infection. Individuals who are coinfecting with HIV/TB should receive treatment for both pathogens because each disease exacerbates one another (Lawn et al., 2009). Further, treating HIV reduces the risk for the development of active TB infection by restoring the host immune system (Lawn et al., 2009).

Globally, one third of children that suffer from malnutrition live in Sub-Saharan Africa (Adler et al., 2017). Ten percent of children under 5 years old die from severe acute malnutrition (SAM) (Adler et al., 2017). Malnutrition is a significant risk factor for development of childhood TB, especially in countries with high rates of TB (Adler et al., 2017). Malnutrition is also associated with fatal outcomes in Mtb-infected children because nutrition plays an integral role in the development of immune responses specific to Mtb (Adler et al., 2017). It is important to take into consideration the implications of malnutrition and the relationship it has on childhood TB and diagnosis.

The BCG world atlas is a database of global BCG vaccination practices which is current as of 2017. All Sub-Saharan African countries currently have national BCG vaccination programs where the first BCG vaccination occurs at birth, with some countries recommending a booster later in life (Zwerling et al., 2011). **Figure 3** shows BCG vaccine policies by country (BCG World Atlas, 2017). Protein used in the BCG vaccine cross-reacts with PPD used in the TST diagnostic tool. High BCG vaccination in Sub-Saharan Africa needs to be taken into consideration when interpreting diagnostic results.

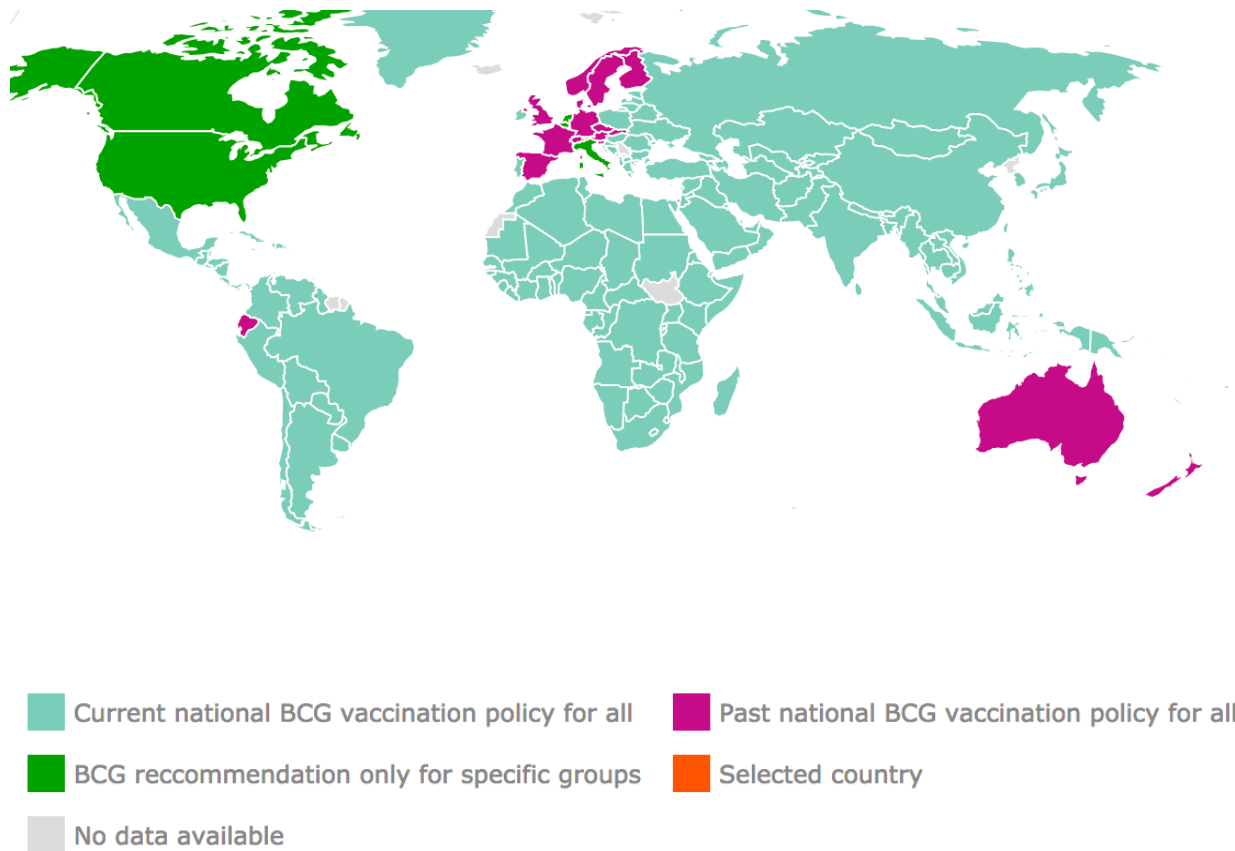


Figure 3. World map of current and past BCG vaccination policies by country

2.0 METHODS

A literature search of the different Mtb diagnostic methods used for children in Sub-Saharan African countries was conducted using the PubMed online scientific database. Different search queries were used including MeSH terms relevant to this topic. MeSH terms included but were not limited to Sub-Saharan Africa OR all countries included in Sub-Saharan Africa, AND Tuberculosis, diagnosis, AND pediatric OR children. **Figure 4** is a screenshot of the history of the query builder in PubMed with every MeSH term included in the search. All searches were filtered by age (birth-18 years) and language (English).

Titles and abstracts were screened first to discard any studies not relevant to the topic of this review. The study had to include children <18 years of age, one of the diagnostic methods discussed in the introduction used to detect TB, and had to be done in a Sub-Saharan African country as defined by the United Nations Development Program (United Nations Development Programme [UNDP], 2018). Studies including adult and child age groups were considered if results/discussions discussed the efficacy of diagnostic tools specifically in the child age groups. A list of all African countries in the Sub-Saharan region are in **Table 1**.

Search	Add to builder	Query	Items found	Time
#6	Add	Search (#3 AND #4 AND #5)	154	10:43:10
#5	Add	Search (Pediatric[tiab] OR Childhood[tiab])	439464	10:22:55
#4	Add	Search ("Tuberculosis/diagnosis"[Mesh] OR "Tuberculosis/diagnostic imaging"[Mesh])	47319	10:22:29
#3	Add	Search (#1 OR #2)	357947	10:21:44
#2	Add	Search (Central Africa[tiab] OR Cameroon[tiab] OR Central African Republic[tiab] OR Chad[tiab] OR Congo[tiab] OR Democratic Republic of the Congo[tiab] OR Equatorial Guinea[tiab] OR Gabon[tiab] OR Sao Tome and Principe[tiab] OR Eastern Africa[tiab] OR Burundi[tiab] OR Djibouti[tiab] OR Eritrea[tiab] OR Ethiopia[tiab] OR Kenya[tiab] OR Rwanda[tiab] OR Somalia[tiab] OR South Sudan[tiab] OR Sudan[tiab] OR Tanzania[tiab] OR Uganda[tiab] OR Southern Africa[tiab] OR Angola[tiab] OR Botswana[tiab] OR Lesotho[tiab] OR Malawi[tiab] OR Mozambique[tiab] OR Namibia[tiab] OR South Africa[tiab] OR Swaziland[tiab] OR Zambia[tiab] OR Zimbabwe[tiab] OR Western Africa[tiab] OR Benin[tiab] OR Burkina Faso[tiab] OR Cabo Verde[tiab] OR Cote d'Ivoire[tiab] OR Gambia[tiab] OR Ghana[tiab] OR Guinea[tiab] OR Guinea-Bissau[tiab] OR Liberia[tiab] OR Mali[tiab] OR Mauritania[tiab] OR Niger[tiab] OR Nigeria[tiab] OR Senegal[tiab] OR Sierra Leone[tiab] OR Togo[tiab])	289087	10:21:04
#1	Add	Search Sub-Saharan Africa	194688	10:20:42

Figure 4. Screenshot of PubMed advanced search builder used to find relevant studies

Table 1. Countries in Sub-Saharan African region as classified by the United Nations

Countries in Sub-Saharan Africa			
Angola	Eritrea	Mauritius	Tanzania
Benin	Ethiopia	Mozambique	Togo
Botswana	Gabon	Namibia	Uganda
Burkina Faso	Gambia	Niger	Zambia
Burundi	Ghana	Nigeria	Zimbabwe
Cameroon	Guinea-Bissau	Rwanda	
Cape Verde	Kenya	Sao Tome and Principe	
Central African Republic	Lesotho	Senegal	
Chad	Liberia	Seychelles	
Comoros	Madagascar	Sierra Leone	
DRC	Malawi	South Africa	
Cote d'Ivoire	Mali	South Sudan	
Equatorial Guinea	Mauritania	Swaziland	

3.0 RESULTS

3.1 DIAGNOSIS OF ACTIVE TB INFECTION

3.1.1 PULMONARY TUBERCULOSIS DISEASE

Bacha et al. (Bacha et al., 2017), did a retrospective descriptive study on children less than 15 years of age in Tanzania who were suspected of active TB infection. Sputum samples were collected and sputum induction was performed on children who were unable to expectorate. Smear microscopy and Xpert were performed on the first sputum sample and culture was performed on a second sample when available. They used clinical diagnosis as a reference standard for Xpert. Sensitivity of Xpert and smear microscopy were 8% and 6% respectively with Xpert only being half as sensitive as culture. This study emphasized the importance of using clinical standards to diagnose TB and that Xpert should not replace culture but instead be used in addition to these other diagnostic algorithms.

Walters et al. (Walters, Demers, et al., 2017) addressed the difficulty of obtaining sputum samples from children suspected of having TB infection and the difficulty in performing sputum induction in resource limited settings. The aim of this study was to see if stool can be used for the rapid identification of TB in children. Children less than the age of 13 years showing signs of TB gave one sputum sample and one stool sample. Smear microscopy, culture, and Xpert were all performed on sputum cultures and Xpert was performed on stool samples. Walters et al. wanted to evaluate the sensitivity and specificity of using a stool sample compared to sputum sample in common diagnostic methods. Using bacteriologic confirmation, stool Xpert had a 31.9% sensitivity and a 99.7% specificity. Stool Xpert only detected Rifampicin resistance in 25% of microbiologically confirmed resistant cases. Stool Xpert was only truly positive in

children with severe clinical disease suffering from comorbidities such as HIV/AIDS and severe acute malnutrition.

In another study, Walters et al (Walters, van der Zalm, et al., 2017) evaluated stool cultures as a replacement to sputum in the detection of TB infection in children. The sensitivity of stool culture in reference to sputum culture yielded a sensitivity of 24%. Testing the same stool sample using Xpert increased sensitivity to 33.3%. As described in (Walters, Demers, et al., 2017), stool Xpert had a higher sensitivity than stool culture (31.9% and 24% respectively). Testing stool by culture enables full drug sensitivity testing whereas Xpert only detects Rifampicin resistance. Walters recommended the usefulness of combining the two testing tools together improving the sensitivity and allowing full drug sensitivity testing. The sensitivity of stool Xpert was found to be higher in this study when compared to other studies that found sensitivities between 15-20%. The difference in stool sample preparation and processing used in previous studies can contribute to the differences in results. Walters concluded that stool culture cannot substitute sputum culture and Xpert for diagnosing childhood TB but can be a useful addition to diagnostic algorithms where sputum cannot be obtained.

Chipindero et al. (Chipinduro et al., 2017) found similar results as Walters et al. (Walters, van der Zalm, et al., 2017). Chipindero et al. used a cross-sectional approach to study 5-16 year olds in Zimbabwe. They tested one sputum sample and one stool sample using Xpert. They used microbiologically-confirmed TB by smear microscopy, culture, and Xpert using sputum as a reference standard. Stool Xpert yielded a sensitivity of 68% and a specificity of 98%. Sensitivity was highest in HIV positive children compared to children not infected with HIV which was found in other studies. The conclusions previously made in stool Xpert studies

were made here. It should be used as an alternative when sputum is unavailable and if possible a second stool sample can significantly increase the sensitivity of the test.

Orikiriza et al. (Orikiriza et al., 2018) did a prospective cohort study on children from one month to fourteen years of age suspected of TB infection at a referral hospital in Uganda. They wanted to compare using sputum and stool specimens with Xpert. Two sputum samples were taken from children in addition to a stool sample. All samples were tested using Xpert, culture, and smear microscopy (except stool samples). Thirty one percent of children participants were also HIV infected. Four percent of children were also microbiologically confirmed to have TB. Sputum Xpert compared to culture had a sensitivity of 90.9% and a specificity of 99.1%. Stool Xpert compared to stool culture had a 55.6% sensitivity and 98.2% specificity. They also assessed patient characteristics associated with positive results. Culture detection yield was more than two times higher in children older than 10 years old and children that provided at least one sputum sample between the ages of 5 and 10 had the highest collection yield. Less than age 5 was associate with the lowest detection yield. This was expected due to the low bacterial load experienced in younger children in addition to the poor quality of samples collected. The results of this study show sputum sample is still better than stool for both Xpert and culture.

Bates et al. (Bates et al., 2013) evaluated the ability of using gastric lavage aspirates in children suspected of having TB as a method for sample using Xpert. Children fifteen years of age or younger in Zambia provided sputum samples by gastric lavage aspiration and sputum induction. Common comorbidities in this sample were malnutrition (22.6%), GI illness (17.9%), and respiratory disorders (34.6%). This study found no significant difference in the sensitivity between sputum and GLA samples using Xpert. Xpert was found to be more sensitive in both sputum and GLA compared to culture. HIV positive children were found to have significantly

lower bacterial loads compared to HIV negative children. Samples from older children yielded higher bacterial loads compared to younger children when looking at TTP in MGIT culture. Researchers concluded that GLA is more valuable than sputum induction because it does not require a professional, proper equipment, or additional staffing and can be readily applied in resource limited settings.

Pohl et al. (Pohl et al., 2016) aimed to evaluate the possibility of using whole blood with Xpert as a sample to detect TB in children. In young children, there is a high prevalence of occult hematogenous spread and disseminated disease and this makes sampling blood a potential approach to detect Mtb in children. Children between 2 months of age and 16 years of age provided a blood AND sputum sample. Less than 50% of children with culture confirmed TB were also HIV infected and 95% of children were BCG vaccinated. Whole blood Xpert had a sensitivity of 7.1% compared to culture confirmed disease. A similar study found similar results that Mtb was rarely found in whole blood of children regardless of HIV status. Small blood volumes (1 mL) were used in this study compared to adult studies which collect 2-20 mLs of blood.

Rose et al. (Rose et al., 2012) evaluated the QuantiFERON Gold TB In Tube performance for diagnosing TB in children less than 15 years of age in Tanzania. Children provided blood for IGRA and HIV testing, and sputum for smear microscopy and culture. TST procedure was done on children as well as a chest x-ray. QFT and TST had sensitivities of 19% and 6% respectively with specificities 90% and 98% respectively. They compared sensitivities of QFT and TST in adults for diagnosing already confirmed TB disease. QFT and TST had significantly higher sensitivities in adults (86% and 89% respectively). Indeterminate diagnostic results were correlated with young age, HIV positivity, and malnutrition status. The study concluded that T-

cell based assays like QFT and TST perform poorly in children with immature or compromised immunity and indeterminate results were a predictor of fatal outcomes in children.

Sekadde et al. (Sekadde et al., 2013) in a cross-sectional study design focused on hospitalized children aged 2 months to 12 years suspected of TB infection. They evaluated Xpert against smear microscopy and culture. Sensitivity of Xpert using culture as a reference was 79.4%. Using clinical diagnosis as a reference, Xpert yielded a 96.5% sensitivity. They also looked at clinical characteristics that were associated with a positive Xpert result. Children older than 5 years of age, having a positive TST result, and a positive TB contact history were all significantly associated with a positive Xpert result. DST was not performed so the sensitivity of Xpert detecting Rifampicin resistance was not calculated. There were significantly higher contaminated culture results when compared to indeterminate results given by Xpert. Sekadde emphasized the importance of clinically evaluating children with negative culture results given the low sensitivity of culture in children and the high probability of TB infection.

A similar study done by Togun et al. (Togun et al., 2015), evaluated Xpert in children less than 15 years of age in an outpatient setting in Gambia. One induced sputum sample was taken from child contacts of TB positive adults and was testing using smear microscopy, culture, and Xpert. Smear and Xpert had sensitivities of 28.6% and 42.9%. There was a 19.4% increase in case detection using Xpert compared to smear microscopy. Using Xpert and culture combined yielded a sensitivity of 32.3% but with no significant difference combining all three methods. With all TB diagnosis and treatment used as a reference, given the low sensitivity culture yields on children, gave an increase of 9.7% to 22.6% compared to culture, smear, and Xpert tests alone.

Lacourse et al. (LaCourse et al., 2014) hypothesized that severely malnourished hospitalized children would have a high prevalence of TB detected by Xpert compared to microscopy and culture methods. Using a prospective observational study design, one induced sputum sample was taken from all children suspected of TB between the ages of 6 months and 60 months of age. Ninety eight percent of child patients were able to provide 2 sputum samples. HIV prevalence in the sample was 18% and 98.3% were BCG vaccinated. Xpert provided 100% sensitivity and 99.6% specificity when compared to culture. Smear only yielded a sensitivity of 50% and specificity of 100% compared to culture. There was a significant higher mortality rate among HIV infected children compared to HIV uninfected children. They emphasize that using confirmed and probable TB cases most likely is more representative of the burden experienced in this group and recommend clinical diagnosis of TB with a low treatment threshold in children living with malnutrition, TB, and HIV.

Zar et al. (Zar et al., 2012) examined the possibility of using nasopharyngeal aspirates (NPA) as a specimen type to diagnose TB in children. They compared NPA and IS samples with smear, culture, and Xpert. In a previous study done by Zar and colleagues, they found that running 2 Xpert tests detected 76% of microbiologically confirmed TB using induced sputum samples. The goal of this study was to compare the diagnostic accuracy of using multiple NPA samples and IS samples with smear, culture, and Xpert. Twenty one percent of children less than fifteen years of age included in this study were HIV infected. Smear was 34.5% sensitive compared to culture and Xpert was 80.5% sensitive compared to culture. Xpert detected 100% of all cases detected by smear. Using culture from either NPA or IS, two Xpert tests on NPA yielded 65.1% and 71.4% with IS. Using both samples, the sensitivity of Xpert increased to 81%. Second samples obtained on a different day were more valuable than samples obtained the same

day hours apart. Because of the low sensitivity of culture, 76% of children were treated for TB based on clinical diagnosis. Zar et al. concluded that Xpert testing on 2 NPA samples should be recommended when IS cannot be performed and culture is not an option.

In a prospective cohort study, Rachow et al. (Rachow et al., 2012) compared smear microscopy, culture, and Xpert. The goal was to explore the performance of Xpert and to look at the impact on time between diagnosis and treatment in children 6 weeks to 14 years of age in a referral hospital. All children were assessed clinically by TST, blood collection, x-rays. Each provided up to 3 sputum samples with IS performed when needed. More than 50% of children enrolled were HIV infected. The study showed that providing second and third samples increased sensitivity by 20% and 13% respectively. Xpert results were not influenced by HIV status. Xpert detected 3 times more culture confirmed cases than smear microscopy. Compared to culture, Xpert had a sensitivity of 66.6%. Xpert detected an additional 16% of TB infected children that were not confirmed by culture but were clinically indicative of TB infection. This study demonstrated the importance of collecting multiple samples when possible and that Xpert could increase TB case identification and decrease time between diagnosis and treatment.

3.1.2 EXTRAPULMONARY DISEASE

Blok et al. (Blok et al., 2014) evaluated the usage of Lipoarabinomannan ELISA for early detection of pediatric TB meningitis. Lipoarabinomannan is an Mtb-specific lipopolysaccharide found in the bacterial cell wall. LAM is released when mycobacteria disintegrate into the bloodstream where it is eventually excreted in the urine. Children from 3 months of age to 13 years of age suspected of TB meningitis were evaluated. Urine was compared to cerebrospinal fluid smear positive and culture positive samples. It was found to have a sensitivity of 4.8% and

a specificity of 93.1%. A higher sensitivity was found in HIV positive children because they tend to have more severe disease with higher bacterial loads and a decreased capacity to contain infection causing the increase excretion of LAM in the urine. They concluded that urinary LAM was of little value for the diagnosis of TB meningitis in children.

Bholla et al. (Bholla et al., 2016) studied children from 8 years of age to 16 years of age in Tanzania to evaluate the use of fine needle aspirates (FNA) and Xpert to diagnose TB lymphadenitis. In two-thirds of cases, children with extrapulmonary TB present with TB lymphadenitis. FNA of lymph nodes were taken from children suspected of TB and analyzed using smear microscopy, liquid culture, cytology, Xpert, and easyNAT. Xpert had a sensitivity and specificity of 56% and 96% compared to standard culture method. This study found a high proportion of invalid results in addition to 48% of the cultures being contaminated. Bholla et al. concluded that Xpert was not an appropriate stand-alone method to diagnose extra pulmonary TB in children and that diagnosis should be done using clinical assessment, culture, and Xpert.

Held et al. (Held et al., 2016) used a prospective study approach to compare Xpert with culture and histology to diagnose pediatric musculoskeletal TB. Children under the age of 13 presenting to a hospital with suspected musculoskeletal TB provided two specimens. One specimen was processed and used for Xpert and the other was used for culture and histology. HIV status was only provided for 29.4% of the children with 10% being HIV infected. Using culture and histology as a reference, Xpert yielded a sensitivity of 73.9% with a specificity of 100%. The sensitivity and specificity of culture against histology was 60.9% and 100% respectively. Smear microscopy had a sensitivity of 60.9% and specificity of 98.8% against culture and histology. Xpert had a significantly shorter turnaround around time compared to culture (0.8 days and 21 days respectively). Xpert detected more cases compared to culture.

Culture detected two cases of multi-drug resistant TB that was not detected by Xpert. The pediatric TB diagnostic algorithm at this hospital in Cape Town, South Africa is to use Xpert and histology together.

Solomons et al. (Solomons et al., 2015) wanted to evaluate using more than one NAAT to diagnose TB meningitis in children ranging from 3 months of age to 13 years in South Africa. They compared Xpert and Genotype assays using CSF of children suspected of TB meningitis. They obtained CSF by using lumbar puncture. When they combined any positive NAAT result it provided a sensitivity of 49%. Smear microscopy and culture using CSF had a sensitivity of 4% and 26% respectively. The two NAATs in addition to the culture had the highest sensitivity of 56% and a specificity of 98% compared to culture alone which was 22% and 100% respectively. They concluded that using NAATs can increase accuracy of diagnosis when performed on CSF in addition to culture.

3.2 DIAGNOSIS OF LATENT TB INFECTION (LTBI)

Adetifa et al. (Adetifa et al., 2010) compared the ability of IGRA and TST to detect latent TB in children. The risk of progression from latent TB to active TB is highest in children and HIV infected children. Adetifa et al. followed 6 month olds to 14 year old children in Gambia that were in contact with TB infected adults. TST was the most responsive to TB exposure compared to the IGRA. Low HIV incidence in Gambia and this sample specifically having low BCG vaccination rates (59.1%) might not be realistic in other Sub-Saharan African countries.

4.0 DISCUSSION

4.1 ACTIVE DISEASE

4.1.1 CULTURE, THE GOLD STANDARD

Culture is still considered the gold standard for detection of TB infection in children. Culture has a low sensitivity of 60% and fails to detect 40% of all infected children (Schumacher et al., 2016). Culture has a long turnaround time of up to 42 days. This increases the time between detection and initiation of treatment. Decision to treat should not only be influenced by a positive culture result. Clinical diagnosis needs to be taken into consideration due to the low sensitivity of culture. The series of studies above compared different diagnostic methods to culture as a gold standard to diagnose pediatric Mtb infection. The studies determining the sensitivity and specificity of culture used clinical diagnosis as a reference.

4.1.2 SMEAR MICROSCOPY

Smear microscopy relies on the presence of acid fast bacilli. Mtb in children is paucibacillary making it more difficult to detect by smear microscopy. As discussed above, smear microscopy is less sensitive in HIV positive and severely ill children (Mosissa et al., 2016). Smear microscopy also does not detect MDRTB delaying treatment until results from DST are available. Every study in this review had low smear microscopy sensitivities compared with other diagnostics. Sensitivity of smear microscopy is lower in younger children compared to older children who present with higher bacterial load.

4.1.3 XPERT MTB/RIF

Ever since the WHO endorsed Xpert MTB/RIF assay in 2011, there have been many studies evaluating its effectiveness against other methods of detection. Xpert has a short turnaround time of 2 hours decreasing the time between diagnosis and treatment. Treatment can be initiated the same day as diagnosis. Xpert was sensitive in HIV infected and malnourished children, which are common comorbidities in high TB burden areas. Xpert simultaneously detects Rifampicin resistance. Xpert is hands-free, thus limiting exposure risks to technologists, is simple to run and interpret results. All studies above evaluating Xpert make the recommendation of using culture, clinical assessment, and Xpert as a diagnostic algorithm to detect childhood TB and that Xpert cannot be used on its own. Studies showed increased sensitivity of Xpert in older children who present with adult-type infection. Similarly, collecting more than one sample for any specimen type also increased sensitivity.

4.1.4 CLINICAL ASSESSMENT

Clinical assessment was found to be important when diagnosing and treating children with TB. Given that culture, the gold standard, still misses a lot of cases, many children are diagnosed clinically without microbiological confirmation.

4.2 LATENT DISEASE

TST and IGRA are the only diagnostics available that detect both active and latent TB infection. Although they detect both, they cannot differentiate between the two forms. There are high BCG

vaccination rates in Sub-Saharan Africa. BCG vaccination cross reacts with the TST diagnostic. IGRAs are more sensitive than TST in children. The study done by Rose et al. showed that the performance of these two tests were affected by the immaturity of the immune system in young children and the impaired immune system experienced in severely ill children (Rose et al., 2012). There are few studies in Sub-Saharan Africa focusing on the diagnosis of latent TB in the pediatric population. Contact tracing studies are costly and require a lot of time to complete. Since the activation of latent TB disease to active TB disease is most common in young and immunocompromised children, latent TB diagnosis and treatment is very important.

The progression of latent TB infection to active TB infection in children can be prevented by contact tracing adults who are Mtb positive. When a child is exposed to Mtb, the five-year risk for developing active TB in children under five years and 5-14 year olds is 33% and 20% respectively (Mandalakas et al., 2015). Further, risk is highest in the first year after exposure and in children who are immunocompromised. Children exposed to TB before age 1 have a 50% chance of developing disease if treatment is not initiated (Mandalakas et al., 2015). If latent disease is detected, there are treatment options to prevent activation into clinical disease. It is difficult to detect latent disease in children in endemic TB areas due to a myriad of reasons. First, children in high endemic areas are BCG vaccinated which causes cross-reactivity with the TST (one of the latent TB diagnostics). In Sub-Saharan Africa, there is a BCG vaccination policy and the majority of children are vaccinated at birth. There is no gold standard for latent TB detection. Most studies use recent contact as a reference standard which can cause either false positive or false negative results. Also, children who are severely ill can have indeterminate diagnostic results and fatal outcomes.

Children in Sub-Saharan Africa suffer from serious comorbidities which can impact interpretation of results.

4.3 USING SAMPLES OTHER THAN SPUTUM

Using samples other than sputum for the diagnosis of pediatric TB is attractive due to the difficulty of obtaining sputum samples from children. Also, disease is paucibacillary and less cavitary in children making detection using sputum a challenge. In this review, sputum (expectorated and induced), NPA, GLA, FNA, whole blood, and urine were all reviewed as possible specimens with culture and Xpert. For extrapulmonary TB, it is more useful to diagnose using specimens specific to the area of infection. A study using CSF as a specimen to diagnose TB meningitis found a sensitivity of 22% for Xpert compared to 24% using culture (Solomons et al., 2015). The low CSF volumes in children made it difficult to obtain enough with culture having a higher sensitivity than Xpert (Solomons et al., 2015). Stool is easy to obtain from children given that that swallowed sputum remains in the stool (Walters, Demers, et al., 2017). The four studies in the review that used stool samples had the limitation of all using different stool processing protocols (Chipinduro et al., 2017; Orikiriza et al., 2018; Walters, Demers, et al., 2017; Walters, van der Zalm, et al., 2017). Stool had a higher yield when used on severely ill children (Chipinduro et al., 2017; Walters, Demers, et al., 2017; Walters, van der Zalm, et al., 2017). It was also found to have an increased sensitivity in children older than 10 years (Orikiriza et al., 2018). It is still not recommended that stool should replace sputum given sputum sensitivity was higher over stool sensitivity using both Xpert and culture. Studies using GLA reported that there needs to be a minimum of 2 samples for it to be effective (Bates et al.,

2013). To diagnose TB lymphadenitis, FNA samples were used. Two thirds of EPTB patients present with lymphadenitis making FNA samples useful (Bholla et al., 2016). Xpert using FNA had a 56% sensitivity compared to culture (Bholla et al., 2016). One study used whole blood as a sample. Whole blood is easy to draw and blood infection is common in disseminated TB infection (Pohl et al., 2016). They found a lower sensitivity when using Xpert compared to culture (7.1%) (Pohl et al., 2016). A limitation was using small blood volumes compared to what is typically used in adults (2-20mLs) (Pohl et al., 2016). Zar et al. compared the sensitivity of NPA and IS specimens and found that two Xpert tests on NPA yielded a sensitivity of 65.1% compared to 71.4% with IS (Zar et al., 2012). They found the incremental value of collecting more than one samples and running multiple tests (Zar et al., 2012). Blok et al. used urine as a specimen compared to CSF for diagnosing TB meningitis and found a low sensitivity of 4.8% using CSF and culture as a reference (Blok et al., 2014).

4.4 LABORATORY AND PERSONNEL CAPACITY

A quality assurance study was done in Ethiopia assessing AFB smear microscopy performances in health facilities using the Ethiopian National Reference Laboratory Guidelines. Forty one percent of laboratories reported back ‘unsatisfactory’ in regards to reading AFB slides correctly (Mosissa et al., 2016). The minimum number of acid fast bacilli needed to produce a positive slide has been estimated to be in between 5,000 and 10,000 bacilli/mL of sputum and if it is below 1,000 the chance of observing it on the slide is less than 10% (Moro et al., 2010). Unreliable lab results can misdiagnose patients with active TB infection delaying treatment and allowing the possibility of transmission to other susceptible individuals. Most countries in Sub-

Saharan Africa rely on sputum microscopy to diagnose TB. In addition to the poor quality of microscopy procedure in low resource settings, children have low bacterial loads making it even more difficult to detect. This is even more so a problem in HIV infected and severely ill children which is endemic in Sub-Saharan African countries.

There is also a lack of prime culture facilities in low resource settings like Sub-Saharan Africa. Culture is still the gold standard to diagnose TB in children and adults. Culture has a long turnaround time of up to 42 days making the time between diagnosis and treatment delayed. A survey done by Saito et al. was administered to 663 health care services in 9 Sub-Saharan African countries to determine the proportion of facilities equipped with proper TB diagnostic tools(Saito et al., 2012). Mtb culture was only available at 53% of health care facilities only reaching 77% of patients (Saito et al., 2012). The survey also found that primary health care services had the lowest access to diagnostics compared to secondary and tertiary services (Saito et al., 2012). Diagnosis and treatment is typically based off of clinical assessment.

A cross-sectional study done in Nigeria examined the gaps in knowledge and training that clinicians experience in management of TB. Ninety four percent of clinicians reported that diagnosis of childhood TB is a problem in Nigeria (Chukwu et al., 2016). Sixty four percent reported having ‘good’ knowledge of TB in children (Chukwu et al., 2016). Out of the clinicians who participated, 13.2% were pediatricians and of those 20.8% were considered specialized in pediatric care (Chukwu et al., 2016). Only half could correctly identify symptoms experienced in TB infected children (Chukwu et al., 2016). Ninety five percent of clinicians agreed that additional training on the use of the TB screening score chart could significantly improve pediatric TB diagnosis (Chukwu et al., 2016). Respondents also reported that childhood TB could be controlled if TB in adults could be improved (Chukwu et al., 2016).

4.5 COMORBIDITIES

Tuberculosis, HIV, and malnutrition are individually associated with high disease burdens in Sub-Saharan Africa. Co-infection exacerbates severe illness experienced in children (Adler et al., 2017). Malnutrition is associated with fatal outcomes in pediatric TB and HIV infected children (Adler et al., 2017). Ten percent of mortality in children under age five is attributed to severe acute malnutrition (Adler et al., 2017). Further, a study found that 10% of HIV positive children on antiretroviral therapy also had severe acute malnutrition (Adler et al., 2017). HIV and malnutrition in children in Sub-Saharan Africa make diagnosing TB even more difficult. The wide spectrum of disease experienced in TB infected children commonly overlap with the signs and symptoms of these other comorbidities (Orikiriza et al., 2018). HIV status was not known in some of the children within these studies. Some parents refused HIV testing for their child and other studies used self-reported measures to identify HIV status. HIV prevalence is also diverse throughout Sub-Saharan Africa with some rates being substantially lower in some countries compared to others. Future diagnostic tools need to take into consideration that TB presentation in children suffering from malnutrition and HIV presents differently than in children without these common comorbidities. These comorbidities are common in TB endemic countries.

5.0 CONCLUSION

The first pillar in the WHO End TB Strategy focuses on early detection, treatment, and prevention for all TB patients (WHO, 2015a). Their aim is to make sure that all patients have equal, unhindered access to affordable care but to also engage in their care. The most important key component of this pillar is early diagnosis of TB including universal drug-susceptibility testing and systematic screening of contact and high-risk groups (WHO, 2015a). In order to diagnose pediatric TB at the earliest stage possible, there needs to be an increase in the use of diagnostic tools that are highly sensitive and specific for TB which are both currently not available due to lack of access and development. Rapid diagnostic tests are not as sensitive in children as they are in adults. New diagnostic tools need to be developed that are sensitive enough to detect pediatric TB disease and take the common comorbidities experienced in high TB burden areas into consideration. Diagnostic tests need to be affordable and practical to those in developing countries like Sub-Saharan Africa. There needs to be a fast turnaround time to decrease the time between diagnosis and treatment. Pediatric TB under detection is a significant public health issue and without sensitive diagnostic tools TB disease will remain endemic. This review of the literature should be interpreted as a guide to increase the efficacy of early diagnostic tools used in the detection of pediatric TB in Sub-Saharan African countries.

APPENDIX: TABLE ON STUDY AND PARTICIPANT CHARACTERISTICS

Table 2. Study and participant characteristics

Author, year	Country	Study Design	Sample Population	Sample(s) Type	% HIV (+)	% BCG Vaccinated	Severe Malnutrition
Bacha et al., 2017	Tanzania	Retrospective Descriptive	<15 years	Sputum	53.50%		30.90%
Walters, Demers, et al., 2017	South Africa	Prospective Cohort	<13 years	Sputum, GA, NPA, stool	13.70%	95%	N/A
Walters, van der Zalm, et al., 2017	South Africa	Prospective Cohort	<13 years	Sputum, GA, NPA, stool	15.4%%	97%	N/A
Chipinduro, Mateveke, Makamure, Ferrand, & Gomo, 2017	Zimbabwe	Cross-sectional	5-16 years	Sputum, stool	51%	N/A	Z-score: -1.09 (-1.99-00.46)*
Orikiriza et al., 2018	Uganda	Prospective Cohort	1 month-14 years				
Bates et al., 2013	Zambia	Prospective Descriptive	<15 years	Sputum, GLA	30.50%	N/A	N/A
Pohl et al., 2016	Tanzania, Uganda	Prospective Cohort	2 months- 16 years	Whole blood, Sputum, GLA, FNA	36%	95%	19%
Rose et al., 2012	Tanzania	Prospective Cohort	<15 years	Sputum, GLA	37%	93%	N/A
Sekadde et al., 2013	Uganda	Cross-sectional	2 months-12 years	Sputum	41.6%	67.60%	27.20%
Togun et al., 2015	Gambia	Prospective Cohort	<15 years	Sputum	0%	72%	29%
LaCourse et al., 2014	Malawi	Prospective Cohort	6-60 months	Sputum	17.6%	98.3%	100%
Zar et al., 2012	South Africa	Prospective Cohort	<15 years	Sputum, NPA	21.9%	N/A	15.60%
Rachow et al., 2012	Tanzania	Prospective Cohort	6 weeks-14 years	Sputum	51.2%	N/A	Z-score: -1.8 (-2.9—0.3)*
Blok et al., 2014	South Africa	Cross-sectional	3 months-13 years	CSF, urine	12.1%	85.7%	N/A
Bholla et al., 2016	Tanzania	Prospective Descriptive	8-16 years	FNA, sputum	20%	N/A	N/A
Held et al., 2016	South Africa	Prospective Cohort	<13 years	Biopsy	10%*	N/A	N/A
Solomons et al., 2015	South Africa	Prospective Cohort	3 months-13 years	Sputum, GLA, CSF	15%	N/A	N/A
Adetifa et al., 2010	Gambia	Retrospective Descriptive	6 months-14 years	Blood	1.10%	59.10%	N/A

*=29.4% of sample known HIV status

*=Weight for age Z-score, classified by WHO

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