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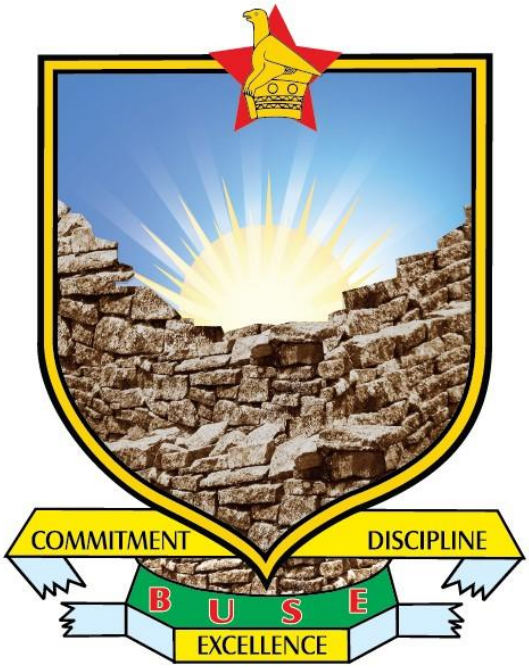

Mycobacterium Tuberculosis (Mtb) community active case findings using point of care tools and centralized laboratory testing to increase active case Tuberculosis Detection.

By

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A research project submitted in partial fulfillment of the requirements for the Bachelor of Science Honours Degree in Biological Sciences.

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APPROVAL FORM

The undersigned certify that they have read the dissertation titled ‘Mycobacterium Tuberculosis (Mtb) community active case findings using point of care tools and centralized laboratory testing to increase active case Tuberculosis Detection’ and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

Signature of student: Nherera Date: 7 June 2024

Signature of Supervisor:  Date: 10/06/24

Signature of Chairperson of Department:  Date: 01/10/24

DECLARATION

I, Kudakwashe Nherera (B200298A) declare that this research herein is my own work and has not been plagiarized from another source(s) without acknowledgement of the concerned author(s) either electronically or otherwise

Signature: Nherera Date: 7 June 2024

Supervisor

I, Dr. P. Jinga, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date: 01/10/24

DEDICATION

This dissertation is lovingly dedicated to my son, who was an unwelcome but precious surprise during my final semester of graduate studies. Though you were still in utero, your presence fueled my determination and motivation to complete this work. Your tiny kicks and flutters were a constant reminder of the beauty and wonder of life, even in the midst of challenging research and long laboratory hours. I hope that one day you will read this work and be proud of the sacrifices we made together to achieve this milestone. Thank you for being my tiny co-author and my constant source of inspiration.

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LIST OF ABBREVIATIONS

ACF: Active case finding

AU: Auramine-O

BCG: Bacilli Calmette-Guérin

BRTI: Biomedical Research and Training Institute

CFU: Colony-forming units

CHW: Community Health Worker

DOT: Direct observation

EVADE-TB: Evaluation of Methods for Tuberculosis Control

IGRA: Interferon-gamma release assay

FN: False negative

FP: False positive

HIV: Human immunodeficiency virus

LJ: Löwenstein–Jensen

LTBI: Latent TB infection

NAAT: Nucleic acid amplification test

NMRL: National Microbiology Reference Laboratory

POC: Point-of-care

QA: Quality assurance

TB: Tuberculosis

TN: True negative

TP: True positive

TST: Tuberculin skin testing

UV: Ultraviolet

WHO: World Health

ZN: Ziehl-Neelsen

ZNTCP: Zimbabwe National TB Control Program

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ABSTRACT

In order to improve TB case detection and treatment initiation among high-risk groups and the general population in Epworth, the study sought to implement and assess the efficacy of a community-based active case-finding strategy between July 2022 and July 2023. The objective is to decrease the transmission and burden of tuberculosis (TB) in the community by detecting and mitigating treatment coverage gaps, improving diagnostic skills, and solving access hurdles. In order to accomplish the primary aim of the study, the following goals guided the research: evaluating the potential impact of active case-finding interventions on the TB epidemiology in Epworth; ascertaining the infection status of TB patients who were screened and enrolled in a TB testing study; and estimating the national prevalence of TB. The research projected a 500-person sample size. 5 true positives, 2 false positives, 31 true negatives, 3 false negatives, and 4 contaminated samples were discovered during the study. Both the centralized laboratory and the point-of-care facility saw comparable results for TB-positive cases. Results were obtained from the Point-of-Care Facility much faster than from the centralized laboratory. Results from the centralized laboratory were returned much more slowly, which caused delays in diagnosis and treatment. Point-of-care facilities have the benefit of producing outcomes quickly. Point-of-care facilities' speedier turnaround times allow for more timely treatment decisions, which are essential for both improving patient outcomes and lowering the risk of tuberculosis transmission. Easy accessibility is an additional benefit. Point-of-care facilities facilitate prompt diagnosis for patients by being more accessible, particularly in underserved or remote areas. Results from centralized laboratories are sometimes delayed. Centralized laboratories' longer turnaround times can impede prompt treatment, which could worsen patient outcomes and increase the risk of transmission. Transportation delays present another difficulty. Sample transportation to central labs may cause delays and raise the possibility of sample contamination, which may affect the precision and dependability of the findings. According to the study, methods for improving follow-up and return rates, especially for centralized lab testing, should be implemented, as well as tactics for improving recruitment to reach the desired sample size.

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CHAPTER 1

INTRODUCTION

1.1 Background

Tuberculosis (TB) is an infectious disease that most often affects the lungs and is caused by bacterium. It spreads through the air when infected people cough, sneeze or spit. Tuberculosis is preventable and curable. About a quarter of the global population is estimated to have been infected with TB bacteria. About 5–10% of people infected with TB will eventually get symptoms and develop TB disease. Those who are infected but not (yet) ill with the disease cannot transmit it. TB is usually treated with antibiotics and can be fatal without treatment. In certain countries, the Bacilli Calmette-Guérin (BCG) vaccine is given to babies or small children to prevent TB. The vaccine prevents TB outside of the lungs but not in the lungs. TB is a major uhealth concern in several countries, and effective diagnostic algorithms for use in human immunodeficiency virus (HIV)-positive patients are urgently needed. Given the global urgency to improve TB case detection, a renewed interest in active case finding (ACF) has risen. Missed TB cases pose a serious threat as they continue to fuel TB transmission in the community.

TB is the leading infectious cause of death worldwide (World Health Organization, 2020). An estimated 3 million people with active tuberculosis were either not diagnosed or were diagnosed but not notified through national reporting systems in 2019 (World Health Organization, 2020). These so-called missing millions of people with undiagnosed or untreated active TB are at risk of death and severe illness and can transmit tuberculosis to others in their households and communities. Declines in global tuberculosis incidence have been slow, and at the rate of current progress, are unlikely to meet the WHO End TB Strategy targets to reduce incidence by 90% and tuberculosis deaths by 95% by 2035 (World Health Organization, 2020). Therefore, the implementation of effective, evidence-based strategies that can increase the diagnosis and treatment of TB, and potentially reduce tuberculosis transmission, are urgently required (Kranzer et al., 2013).

1.2 Justification

Community-based active case-finding interventions might identify and treat more people with TB than standard case detection. The barriers to access diagnosis and receive treatment, in addition to insufficient case identification and reporting, lead to TB spreads in communities, especially among hard-to-reach populations. This project is to describe and evaluate a community-based ACF strategy for the detection of tuberculosis cases among high-risk groups and general population in Epworth from July 2022 to July 2023.

1.3 Statement of the Problem

Tuberculosis (TB) remains a significant public health challenge, particularly in communities with limited access to healthcare services. Traditional methods of TB case detection often fail to reach all individuals, especially those in hard-to-reach or marginalized populations. Barriers such as limited healthcare infrastructure, social stigma, and lack of awareness contribute to insufficient case identification and reporting. Consequently, TB continues to spread within these communities, exacerbating the public health burden. This project seeks to address these challenges by implementing and evaluating a community-based active case-finding (ACF) strategy for the detection of TB cases among high-risk groups and the general population in Epworth from July 2022 to July 2023.

1.4 Aim

The aim of the project is to implement and evaluate the effectiveness of a community-based active case-finding strategy for improving TB case detection and treatment initiation among high-risk groups and the general population in Epworth from July 2022 to July 2023. This involves identifying barriers to access, enhancing diagnostic capabilities, and improving treatment coverage to ultimately reduce the transmission and burden of TB in the community.

1.5 Objectives

The objectives of the study were to

To assess whether active case-finding interventions can affect TB epidemiology in Epworth.

To determine infection status in TB screened and enrolled participants in a TB testing study.

To estimate the prevalence of TB at the national level.

1.6 Limitations

GeneXpert and culture require functional lab infrastructure and reliable electricity/supplies which may not be consistently available at point-of-care. Sputum sample quality could impact test accuracy and variability between microscopy, culture and GeneXpert results. Drug susceptibility testing requires specialized equipment and training which may not be readily available.

1.7 Delimitations

The study will be conducted only in Epworth, Harare, Zimbabwe. Only sputum samples will be collected and tested for TB diagnosis using GeneXpert, smear microscopy and culture. Drug susceptibility testing will only be performed on samples confirmed positive for TB via GeneXpert or culture. High-risk community determination will use TB prevalence data from the study sites only in statistical analysis. Cross-sectional design limits inferences to the specific time period of the study.

1.8 Definition of Terms

Epidemiology

The study and analysis of the distribution, patterns, and determinants of health and disease conditions in specified populations. It forms the foundation for public health interventions aimed at improving health outcomes and preventing disease. Epidemiology is used to understand the spread and control of tuberculosis within the study population, guiding targeted interventions (Last, 2001).

Latent Infection

An infection in which the pathogen persists in the host without causing overt symptoms or active disease but can reactivate and cause illness under certain conditions. Identifying and addressing latent TB infections is crucial for preventing the reactivation and further transmission of tuberculosis (Kumar, Abbas, & Aster, 2017).

Case Load

The number of cases of a particular disease or medical condition being managed by a healthcare provider or institution within a specific period. Assessing the case load helps evaluate the healthcare system's capacity to manage TB and informs resource allocation (Thrusfield, 2018).

Prevalence

The proportion of a population found to have a particular disease or condition at a specific time or over a specified period. Understanding the prevalence of TB in the community helps in planning and prioritizing public health interventions (World Health Organization, 2014).

CHAPTER 2

LITERATURE REVIEW

2.1 Etiology of Tuberculosis

TB is caused by a bacterium called *Mycobacterium tuberculosis*. The bacteria usually attack the lungs, but it can attack any part of the body such as the kidney, spine, and brain. Not everyone infected with TB bacteria becomes sick. As a result, two TB-related conditions exist: latent TB infection (LTBI) and TB disease. If not treated properly, TB disease can be fatal (Steingart et al., 2014).

2.2 History of Tuberculosis

On March 24, 1882, Dr. Robert Koch announced the discovery of *Mycobacterium tuberculosis,* the bacteria that causes tuberculosis (TB). During this time, TB killed one out of every seven people living in the United States and Europe. Dr. Koch's discovery was the most important step taken toward the control and elimination of this deadly disease. A century later, March 24 was designated World TB Day: a day to educate the public about the impact of TB around the world. Until TB is eliminated, World TB Day won't be a celebration. But it is a valuable opportunity to educate the public about the devastation caused by TB and how it can be stopped. *Tuberculosis* (TB) was called "phthisis" in ancient Greece, "tabes" in ancient Rome, and "schachepheth" in ancient Hebrew. In the 1700s, TB was called "the white plague" due to the paleness of the patients. TB was commonly called "consumption" in the 1800s even after Schönlein named it *Tuberculosis*. During this time, TB was also called the "Captain of all these men of death." During the Middle Ages, TB of the neck and lymph nodes was called "scrofula." Scrofula was believed to be a different disease from TB in the lungs. Today, our names for TB tell us where TB is located (pulmonary, extrapulmonary) and how to treat it (drug-susceptible, drug-resistant, multidrug resistant, and extensively drug-resistant). CDC and many organizations around the world are working towards a future where we call TB "history" (Prabh & Singh, 2019).

2.3 Tuberculosis in humans

TB in humans in Zimbabwe can be traced back thousands of years, however routine surveillance was established more recently. In the 1990s, Zimbabwe began facing a significant TB epidemic with rising case notifications due to the HIV/AIDS crisis (Takarinda et al, 2017). By 1997, national TB prevalence reached 692 per 100,000 population?, one of the highest in the world at the time (Maher et al., 1998). In 2006, the national TB control program embarked on innovative research interventions in collaboration with international partners (Takarinda et al., 2017). A sample vital registration system with verbal autopsies was implemented under the ZIMPHIA survey to generate mortality data for guiding health priorities (Gwitira et al, 2021). Active case finding initiatives focused on hospitalized patients and PLHIV contacts also expanded (Takarinda et al., 2017). Most recently in the 2017 national TB prevalence survey, Zimbabwe adopted World Health Organization recommendations for latent TB infection screening among high-risk groups, given estimates indicating over 50% of the population has been infected with Mycobacterium tuberculosis at some point (Timire et al, 2019). Ongoing research aims to assess interventions to prevent TB activation and achieve the End TB Strategy goals in Zimbabwe (Dlodlo et al., 2017).

Transmission

Before the discovery of the bacteria that causes TB, the disease was thought to be hereditary. In the early 1800s, there were “vampire panics” throughout New England. When a TB outbreak occurred in a town, it was suspected that the first family member to die of TB came back as a vampire to infect the rest of the family (Krishna, 2022). To stop the vampires, townspeople would dig up the suspected vampire grave and perform a ritual. On March 24, 1882, Robert Koch announced his discovery that TB was caused by a bacterium in his presentation “Die Aetiologie der Tuberculose” at the Berlin Physiological Society conference. The discovery of the bacteria proved that TB was an infectious disease, not hereditary. In 1905, Koch won the Nobel Prize for Medicine and Physiology. Today, TB is known as an airborne infectious disease, spread when a person with TB coughs, speaks, or sings. When a person is diagnosed with TB, a contact investigation is done to find and test people (like family members) who may have been exposed to TB. People diagnosed with TB or latent TB Infection are then treated (Krishna, V. (2022).

TB is transmitted from an infected person to a susceptible person in airborne particles, called droplet nuclei (Naik & Selvaraj, 2022). These are 1–5 microns in diameter. These infectious droplet nuclei are tiny water droplets with the bacteria that are released when persons who have pulmonary or laryngeal tuberculosis cough, sneeze, laugh, and shout. These tiny droplet nuclei remain suspended in the air for up to several hours. *Mycobacterium tuberculosis* is transmitted through the air, not by surface contact. This means touching cannot spread the infection unless it is breathed in. Transmission occurs when a person inhales droplet nuclei containing bacteria. These droplet nuclei travel via mouth or nasal passages and move into the upper respiratory tract. Thereafter they reach the bronchi and ultimately to the lungs and the alveoli. Symptoms of TB include coughing that lasts for more than three weeks, hemoptysis, chest pain when breathing or coughing, weight loss, fatigue, fever, night sweats, chills, and loss of appetite (Gombe et al., 2019). *M. tuberculosis* also has the potential of spreading to other parts of the body. This can cause blood in urine if the kidneys are affected, and back pain if the spine is affected (Streicher, 2020).

2.4 Methods of detection and diagnosis

2.4.1 Microscopy

Other bacteria are commonly identified with a microscope by staining them with Gram stain. However, the mycolic acid in the cell wall of *M. tuberculosis* does not absorb the stain. Instead, acid-fast stains such as Ziehl–Neelsen stain, or fluorescent stains such as auramine are used. Cells are curved rod-shaped and are often seen wrapped together, due to the presence of fatty acids in the cell wall that stick together. This appearance is referred to as cording, like strands of cord that make up a rope. *M. tuberculosis* is characterized in tissue by caseating granulomas containing Langhans giant cells, which have a "horseshoe" pattern of nuclei (Acharya et al, 2020).

2.4.2 Culture

Slant tubes of Lowenstein-Jensen medium. From left to right:

Negative control

- M. tuberculosis: Dry-appearing colonies

- Mycobacterium avium complex: Wet-appearing colonies

- M. gordonae: Yellowish colonies

Culture Techniques for Isolating and Identifying M. tuberculosis

Laboratory cultivation of Mycobacteri um tuberculosis is necessary for definitive diagnosis of active tuberculosis (TB) disease, particularly among smear-negative and extrapulmonary cases, as well as precise drug sensitivity testing to delineate optimal antibiotic therapy. Compared to other common bacterial pathogens, M. tuberculosis is exceptionally challenging to propagate in-vitro given complex growth requirements and characteristically slow doubling time of ~24 hours (Chegou et al., 2019). Accordingly, specialized media formulations and incubation conditions up to 6-8 weeks are imperative to support visible colony development.

Culture Media Options

Egg-based solid formulations containing glycerol and malachite green selective inhibitors such as Löwenstein–Jensen (LJ) remain the most accessible option globally (Singhal et al., 2020). With appropriate M. tuberculosis inoculation either directly from specimens or following decontamination, observation of rough, crumbly, off-white granular colonies confirm growth at 35-37° Celsius over 3-8 weeks. Though cost-effective with minimal biosafety requirements, LJ notoriously suffers from fungal overgrowth and lower M. tuberculosis yields around 105 colony-forming units (CFU) per ml (García-Basteiro et al, 2018). To enhance sensitivity and purity, while shortening turnaround time to 1-3 weeks, automated liquid culture systems are considered gold standard despite infrastructure demands. Flagship platforms Middlebrook 7H9/7H12 broth offer optimized protein, catalase, and fatty acid energy sources in a gentle orbital shaking incubator (35°C) set-up (Kruh-Garcia et al., 2014). Positive growth triggers either fluorescent sensor detection or measured pressure changes from ongoing M. tuberculosis respiration. However, contamination troubleshooting and higher biosafety precautions around aerosol generation remain challenges.

Middlebrook 7H10/7H11 agar permits more specific colony morphology assessment akin to conventional solid media, though liquid cultures are ultimately quantified using most-probable

number statistical principles given dispersed micro-colonies rather than countable discrete colonies. Selective antimicrobials like polymyxin B, amphotericin B and trimethoprim can be incorporated to minimize contaminants across all media types without affecting mycobacterial recovery (Singhal et al., 2020). But specialized training is imperative to distinguish true positives from breakthrough non-tuberculous environmental mycobacteria given similarly slow generation times.

Confirmatory Identification Tests for M. tuberculosis

Several macroscopic, microscopic and biochemical features distinguish M. tuberculosis from other pathogens once growth occurs. It's rough, crumbly colony texture with folded appearance and non-pigmented off-white color is fairly characteristic, enabled by its wax-rich cell envelope structure primarily containing mycolic acids (MacLean et al, 2020). Microscopically following Ziehl-Neelsen staining, red acid-fast retaining bacilli visible under oil immersion light microscopy confirms mycobacterial genus identity, though other ancillary testing is essential to specify M. tuberculosis species.

High performance liquid chromatography detection of the unique lipid-rich cell wall component tuberculosteric acid present solely in M. tuberculosis can provide species-level identification within a day (Walzl et al, 2018). Genotypic assays like the Hain Line Probe and Xpert MTB/RIF complementary DNA probes have replaced conventional biochemical testing for niacin accumulation, nitrate reduction, and catalase thermostability to enable rapid confirmation once indicator growth is observed (Walzl et al, 2018). Matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry platforms that generate unique proteomic spectra fingerprints are also emerging for both species' delineation and antimicrobial resistance markers detection in under three hours. Taken together, the series of selective media culture in automated or solid formats coupled with an array of modern confirmatory assays facilitate unambiguous laboratory detection of M. tuberculosis. Though still a slower process than routine bacteria given complex nutritional requirements, advances in culture-based diagnostics have enhanced sensitivity to 84-90% over direct sputum testing while revealing drug susceptibility profiles to pinpoint appropriate antibiotics for prompt cure (Singhal et al., 2020). Inclusion among frontline molecular assays and sound biosafety precautions for targeted testing remain pivotal for supporting tuberculosis elimination goals.

2.5 Point of care Tuberculosis diagnosis

The POC investigational assays will be measured against a composite diagnostic algorithm comprising symptoms, sputum GeneXpert Ultra, TB microscopy and TB culture results. Potential outcomes are Definite TB, Probable TB, Possible TB, and No TB. Participants classified as Definite TB or Probable TB will be regarded as gold standard positive, and participants with No TB will be regarded as gold standard negative. Definite TB denotes sputum culture or GeneXpert Ultra confirmation; Probable TB is a combination of radiological or microbiological; and No TB indicates that all tests are negative for TB and that an alternative diagnosis exists. Possible TB occurs in cases with contradicting evidence, often due to loss to follow-up. This category is excluded from analysis because of the uncertainty of TB (Valvo J, et al. 2017).

2.6 Factors Which Increase the Risk of Becoming Infected

2.6.1 Infancy and pubescent children

Infants, with their weaker immune systems, can easily succumb to TB if they become infected. These children are at a greater risk of suffering serious complications and thus require special attention.

2.6.2 Stress

It is believed that stress and irregular lifestyle patterns can also make one more susceptible to TB.

2.6.3 Gender Differences

Women are more susceptible to the disease before middle age, and men become more susceptible after reaching middle age.

2.6.4 Diabetes, Stomach Ulcers, and Other Conditions

People with diabetes or stomach ulcers, or those who have had a gastrectomy, are known to be more susceptible to TB. In addition, those with pneumoconiosis, those who have undergone

intestinal bypass operations, those undergoing dialysis, or those with hemophilia are also said to be vulnerable.

2.6.5 Adrenocortical Hormone Agents and Biological Drugs

Anti-cancer drugs and adrenocortical hormone agents (steroids) used to treat asthma, collagen disease, cancer, and other disorders can weaken the immune system and increase the risk of contracting TB. People using TNFα inhibitors (biological drugs), currently receiving attention as a treatment for rheumatoid arthritis and other disorders, have also been recognized worldwide as being more susceptible to the disease.

2.6.7 AIDS, HIV Infection

In Africa and some Asian countries, the presence of populations with weakened immune systems due to HIV, has led to increases in the number of TB infections, and become a serious issue.

2.6.8 Hereditary Predisposition

Science is gradually uncovering information indicating that resistance to TB is genetically determined.

2.6.9 Tobacco and Other Factors

Smokers, people who have contracted tuberculosis in the past, people who have not received the BCG vaccination and received positive results from tuberculin tests, people with others close to them who have recently contracted TB, and individuals in similar circumstances are also at a higher risk of contracting the disease (Chegou NN, et al. 2018).

2.7 Scientific classification of Tuberculosis

Domain	Bacteria
Phylum	Actinobacteria
Class	Actinobacteria
Order	Actinomycetales

Family Mycobacteriaceae

Genus Mycobacterium

Species *Mycobacterium tuberculosis*

Binomial name *Mycobacterium tuberculosis*

2.8 Characteristics of the Genus

Mycobacteria are members of the order Actinomycetales, and the only genus in the family Mycobacteriaceae. Currently, the genus *Mycobacterium* has more than 100 recognized or proposed species, including numerous pathogens and saprophytic organisms of warm-blooded animals. The distinguishing characteristics of this genus include acid-fastness and the presence of mycolic acids. Mycobacteria are slender, non-spore-forming, rod-shaped, aerobic, slow-growing, and free-living in soil and water (Natarajan et al, 2020). These bacteria have a generation time of about 20 h, thus isolation and identification may take up to 6 weeks (although a few species may grow in only 5–7 days). These bacteria are acid-alcohol-fast, which means that after staining they resist decolorization with acidified alcohol as well as strong mineral acids. The property of acid-fastness, resulting from waxy materials in the cell walls, is particularly important for recognizing mycobacteria. The staining procedures must be carefully performed because other Gram-positive bacteria (e.g., Nocardia, Corynebacterium, and Rhodococcus) are often partially acid-fast (Natarajan et al, 2020).

2.9 Tuberculosis in Zimbabwe

Tuberculosis (TB) remains one of the top ten causes of morbidity and mortality in Zimbabwe, as a major public health threat exacerbated by the high HIV/AIDS burden nationwide (Chatora et al., 2019). Zimbabwe reports over 20,000 TB cases annually, with 72% among people living with HIV per the 2017 TB prevalence survey data (Matambo et al, 2020). The TB/HIV co-epidemic drives Zimbabwe's TB incidence rate upwards of 200 per 100,000 population based on case notification trends in recent years (Takarinda et al., 2015). Ongoing research and control efforts aim to curb the dual infections in alignment with global End TB targets of reducing TB deaths by 95% and cutting new cases by 90% between 2015 and 2035 (Ncube et al., 2017).

2.9.1 TB Prevention and Control History in Zimbabwe

Prior to coordinated surveillance and reporting structures, estimates relied on hospital-based data subject to gross underestimations of the nationwide TB caseload (Maher et al., 1998). By the 1990s however, Zimbabwe began facing a significant TB crisis with escalating case notifications largely attributed to the expanding HIV epidemic (Tarumbiswa, 2019). Recognizing the threat early on, the Zimbabwe National Tuberculosis Control Programme (ZNTCP) joined the World Health Organization (WHO) DOTS strategy in 1994 for enhanced case detection and treatment adherence support via direct observation of therapy (Chirenda et al., 2003). By 1997, national TB prevalence reached 692 per 100,000 - among the highest globally at the time (Maher et al., 1998) given complications of drug-resistant TB and HIV coinfections (Tagwireyi et al., 2016).

In 2006, the ZNTCP embarked on innovative research collaborations towards strengthening the coordination, evidence-based policies, and impact of TB control interventions through Evaluation of Methods for Tuberculosis Control (EVADE-TB) initiatives across 13 high-density communities (Takarinda et al., 2015). The EVADE-TB platform investigated active case finding coupled with intensified case holding for vulnerable groups, yielding 51% and 68% higher case notification rates among contacts of TB patients and HIV clinic attendees respectively (Takarinda et al., 2016). Building on these approaches, the national TB prevalence survey adopted household contact investigation coupled with evaluation of latent tuberculous infection using interferon-gamma release assays (IGRAs) and tuberculin skin testing (TST) among high-risk groups (Chatora et al., 2019). IGRA introduction aims to improve detection and preventive therapy eligibility for 30% of household contacts estimated to have latent TB based on historic TST conversion proportions (Benede et al., 2018).

Beyond active case finding, the ZNTCP has collaborated with the Zimbabwe Population-based HIV Impact Assessment (ZIMPHIA) 2015-16 survey team to institute a nation-wide sample vital registration system leveraging mobile technology and verbal autopsy questionnaires for real-time mortality surveillance (Gombe et al., 2019). Cause-specific mortality data coupled with case notification trends will strengthen the evidence guiding control prioritizations for targeted impact across populations (Gombe et al., 2020). Ongoing initiatives also seek to decentralize state-of-the-art rapid diagnostics, consolidate specimen referral linkages, expand drug sensitivity testing for emerging drug-resistant TB, optimize HIV/TB co-treatment service delivery models, and

investigate socio-behavioral interventions to improve case detection and treatment adherence (MOHCC & NTLP Zimbabwe, 2020).

2.9.2 Current Practices in TB Identification, Diagnosis and Patient Management

Per National Tuberculosis Management Guidelines, all individuals presenting to health facilities with cough lasting two or more weeks must be evaluated for presumptive TB (MoHCW Zimbabwe, 2021). Using a standard symptom screening form, frontline healthcare workers assess risk and collect sputum samples from suspects for laboratory testing. However, intensified case finding has demonstrated that complementing facility-based passive screening with active surveillance among hospitalized patients and household contact investigations substantially increases case detections by 45-90%, necessary for epidemic control (Takarinda et al., 2020).

The standard for laboratory confirmation remains sputum smear microscopy and culture given resource limitations, though bleach concentration to improve low bacterial load detection and drug susceptibility testing is conducted at provincial centers and the National Microbiology Reference Laboratory (NMRL) (NAC Zimbabwe, 2020). Introduction of rapid molecular GeneXpert MTB/RIF and Truenat assays at centralized laboratories has enhanced case detection among smear-negative and rifampicin resistant TB, though accessibility constraints limit widespread use (MOHCC & NTLP Zimbabwe, 2020). Decentralizing capacity for updated WHO-recommended diagnostic algorithms is essential for timely and accurate detection, particularly among PLHIV co-infected patients (Tagwireyi et al., 2016).

Upon diagnosis, standardized treatment commences with a 6-month antibiotic regimen containing rifampicin served via direct observation (DOT) to ensure adherence and prevent resistance (Bandason et al., 2018). For drug-sensitive TB, the intensive phase consists of daily isoniazid, rifampin, pyrazinamide and ethambutol for two months, followed by four months of isoniazid and rifampin continuation phase (Mungofa et al., 2007). DOT provision through integrated community and home-based care has proven highly effective for vulnerable groups, achieving treatment success rates over 80% (Bandason et al., 2013).

However, for rising MDR- and XDR-TB cases, expanded surveillance with routine drug sensitivity testing and centralized database tracking is needed to quantify resistance patterns and

inform optimal second-line treatment protocols (NAC Zimbabwe, 2020). Improving MDR-TB diagnosis and case management under programmatic management of drug-resistant TB guidelines is vital, though constrained by prohibitive costs of long regimens and isolation requirements (MOHCC & NTLP Zimbabwe, 2020). Ongoing zoning initiatives to map resistance profiles across provinces will further equip evidence-based policies for tailored MDR interventions (Ndlovu et al, 2018).

2.9.3 Research Priorities for Elimination

While laudable treatment success has led to stable incidence rates in recent years (Benede et al., 2018), Zimbabwe is lagging in meeting the steep End TB Strategy targets. Key research priorities for acceleration include assessing efficacy and scaled implementation feasibility of state-of-the-art molecular diagnostics to rapidly identify 90% of estimated incident cases and drug susceptibility profiles (Ndlovu et al, 2018). Enhanced surveillance through the in-development electronic TB registry dashboard will strengthen real-time case tracking, cohort analyses, and programmatic indicator reporting for targeted response (MOHCC & NTLP Zimbabwe, 2020). Ensuring integrated people-centered TB/HIV services and decentralizing access is also vital for early detection and prevention of complications (Furara et al., 2019). Social and structural interventions to address stigma-related barriers limiting healthcare engagement require ongoing operational research (Tshuma, 2019). With continued partnerships, evidence generation for innovation, and policies addressing evolving demographic needs, elimination goals can be achieved in Zimbabwe.

2.10 Importance of active case finding in Zimbabwe

Tuberculosis (TB) remains one of Zimbabwe's most urgent public health threats, compounded by the unprecedented TB/HIV co-epidemic with 72% of TB patients co-infected nationally (Benede et al., 2018). Despite a well-established passive case finding and DOTS treatment program under the Zimbabwe National TB Control Program (ZNTCP), persistent diagnostic delays and asymptomatic transmission continue driving Zimbabwe's TB incidence rate over 200 per 100,000 population in recent years (Takarinda et al., 2015). Achieving ambitious End TB targets of reducing TB mortality by 95% and incidence by 90% between 2015 and 2035 is

increasingly contingent on earlier case detection and rapid initiation of treatment to curb ongoing transmission (Tshuma, 2019). A growing body of evidence demonstrates that passive facility-based screening vastly underestimates the prevalence of undiagnosed TB in communities, amounting to a lost opportunity for interrupting infection spread (Chikovore et al., 2015).

2.10.1 Magnitude of Underdiagnosis - TB Prevalence Survey Findings

The inaugural 2017 national TB prevalence survey provided pivotal insights on the disconnect between presumed versus actual TB caseload based solely on routine surveillance data. Through cluster-based sampling of over 13,000 households, investigators found 75% of the 3380 bacteriologically positive cases identified had no prior TB treatment history or current care seeking for related symptoms (Zarova et al., 2018). This amounted to over 21,000 cases missing from national statistics and notifications - a 3-fold higher TB prevalence than past guesses presumed using passive data alone (Chatora et al., 2019). The findings mirror trends across sub-Saharan African prevalence surveys whereby 40-60% of prevalent TB cases in communities have not been engaged by care systems (WHO, 2013).

2.10.2 Persistent Barriers to Access and Diagnosis

Mycobacterium tuberculosis transmission is notably driven by the pool of undiagnosed cases, whereby an untreated active case can infect 10-14 others annually, perpetuating avoidable incidence (Macheker et al, 2019). Zimbabwe's entrenched healthcare access barriers around stigma, user fees, transport costs, informal care preferences, and diagnostic challenges continue delaying diagnosis by a month on average even for those who actively seek care (Dlodlo et al., 2017). As a case in point, the 2017 TB Patient Cost Survey found 65% of patients remain AFB sputum positive for MTB after an average 4-week diagnostic delay, suggesting ongoing transmission risk (TB CARE II Zimbabwe, 2017).

From the healthcare provider perspective as well, diagnostic capacity constraints with suboptimal specimen collection, inadequate index of clinical suspicion among frontline workers, lack of systematic contact investigation procedures, and infrastructure limitations diminish case finding effectiveness (Mabuto et al., 2014). Such multifactorial delays coupled with asymptomatic

transmission drive underestimated caseload and stifled progress towards elimination goals (Chikovore et al., 2015).

2.10.3 Rationale and Evidence on Active Case Finding Strategies

Active case finding (ACF) has thus emerged as a vital complementary approach to curb ongoing transmission, prevent diagnostic delays, and increase case detection through systematic screening of high-risk groups via outreach in communities, workplaces, congregations, correctional facilities and targeted testing of patient contacts (Shamu et al., 2016). Patients diagnosed via active modalities are also less likely to abandon treatment given community-based accompaniment support structures (Takarinda et al., 2020).

Zimbabwe is among many TB high-burden countries adopting WHO's recommendation to integrate intensified ACF initiatives alongside passive case finding for comprehensive and prompt diagnosis (NAC Zimbabwe, 2020). Programmatic ACF data has consistently revealed high yields, with around 4% of contacts testing positive (Takarinda et al., 2016) and even higher 6-10% yields from community or home-based contact investigation employing mobile vans and expanded screening (Takarinda et al., 2020; Macheker et al, 2019). Such community-wide systematic initiatives have boosted case findings and notifications by 45-90% over standard passive facility detection in both local and regional contexts (Takarinda et al., 2016; Claassens et al., 2013).

With asymptomatic disease reservoirs comprising the bulk of prevalent cases, expanded screening and chest radiographic evaluation among risk groups have proven high-yield, cost effective at USD $105-$477 per case detected, and necessary for curbing transmission earlier (Kranzer et al., 2013; van't Hoog et al., 2013). Targeted testing initiatives focusing on hospitalized patients, people living with HIV (PLHIV), diabetics, miners, prisoners, and congregate settings have yielded 4 times more cases over routine practice (Getahun et al., 2010; Kranzer et al., 2010). When coupled with treatment initiation within two weeks of diagnosis, over 2-fold higher patient recovery and 50% reduced TB mortality is achievable (Churchyard et al., 2015). Such empirical evidence affirms intensified ACF as vital to reaching End TB targets.

2.10.4 Gaps in Implementation

While showing vast promise, available data also indicates missed opportunities around suboptimal screening coverage during contact investigations (only 60% of household members evaluated), infrastructure bottlenecks, and funding constraints limiting community health worker capacity for systematic household evaluation in remote areas (Takarinda et al., 2020; Mangwanya et al., 2020). Developing operational guidance and context-specific protocols is thus essential to close system-level gaps for translating policy commitments on ACF into impactful practice (van Hoog et al., 2020.

As ACF further scales up nationally, our proposed impact evaluation of ongoing case finding initiatives and their yield based on programmatic data aims to quantify incremental gains on case notifications and treatment outcomes. Findings will directly highlight high-yield risk groups and operational procedures for optimizing early diagnosis. Cost-effectiveness assessments of varied ACF modalities will also guide strategic budget allocations towards sustaining scale-up. Such implementation-oriented evidence can accelerate Zimbabwe's progress towards bold mortality and incidence reduction goals that mathematical models confirm are unattainable relying solely on status quo passive case finding (Knight et al., 2015).

With continued commitment towards an evidence-based ACF offensive targeting high transmission zones, Zimbabwe can get back on track to end its longstanding TB epidemic, saving tens of thousands of lives and billions in health system costs annually. No one with TB should have to suffer without timely diagnosis and prompt cure being accessible. What barriers remain are surmountable with backed political will and coordinated action centered on communities' needs.

CHAPTER 3

MATERIALS AND METHODS

3.1 Point of Care (Study Area)

An evaluative study was conducted of decentralized point-of-care (POC) testing for tuberculosis between July 2022 to July 2023 in Epworth, a high-density suburb located approximately 10 km? outside Harare, Zimbabwe. Epworth has a population of over 200,000 people residing in just 10 square kilometers, reflecting an average population density of over 20,000 people per square kilometer. The majority of residents live in overcrowded, makeshift housing with poor ventilation and sanitation systems, posing immense health risks and increased probability of *Mycobacterium tuberculosis* transmission.

Routine surveillance data estimated Epworth's annual tuberculosis notification rate exceeded 350 cases per 100,000 population?, well above Zimbabwe's national incidence level of 330 per 100,000 in 2018. However, local diagnostics were constrained without sufficient staff or infrastructure. The two primary clinics struggled to meet testing and treatment demand, with frequent equipment breakdowns and long wait times. Thus, aimed to introduce the GeneXpert system for rapid molecular testing at the point-of-care to accelerate case detection and strengthen existing microscopy services. Four GeneXpert modules were deployed to the two clinics under my study protocol. Assisted with active case finding and sample transportation. Rigorous evaluation of this intervention provided valuable evidence to guide scale-up decisions. If shown effective, similar approaches could help reduce the global disease burden through improved access to quality diagnostics.

3.2 Study design

A cross-sectional experiment conducted.

A prospective cross-sectional design was utilized to evaluate the diagnostic accuracy and operational impact of decentralized point-of-care testing with GeneXpert MTB/RIF in the high TB burden setting of Epworth, Zimbabwe. Ethical approval was obtained from the Medical Research Council of Zimbabwe under BRTI, and all participants provided written informed consent. The study period ran from July 2022 through July 2023 to ensure capture of seasonal variations in tuberculosis transmission and diagnosis. Individuals were recruited through active case finding strategies combining both community outreach and enhanced clinic-based screening of presumptive TB patients. Standardized screening tools are recommended by the WHO and Zimbabwe NTLP to systematically identify individuals likely to harbor active TB disease among those presenting for care or encountered in community settings (WHO, 2013). Accordingly, Community Health Workers (CHWs) and clinic nurses were trained on administration of a structured symptom questionnaire involving common pulmonary TB symptoms - current cough, fever, night sweats, weight loss, chest pain or breathlessness. The questionnaire was administered verbally in local languages to consenting adults presenting at Epworth Polyclinic as well as those visited door-to-door by CHWs during planned community outreach campaigns with support from the Epworth Local Board. Screening was offered regardless of known TB contact history or HIV status to capture both presumptive secondary cases and new incident infections per national guidelines. Individuals reporting one or more TB symptoms were classified as pulmonary TB suspects according to standardized definitions and requested to self-collect up to three sputum samples over the subsequent 48 hours for laboratory evaluation (Zhu et al., 2021). To enhance diagnostic yield, participants were coached through breathing techniques to generate deeper coughs able to bring up mucus from lower respiratory compactions using short video instructions on mobile tablets in local languages (Gegia et al., 2017). Samples were expectorated into sterile 50ml screw-cap containers which CHWs directly observed being properly sealed before labeling with participant identifiers. Cold chain conditions were maintained during transportation by keeping specimens on ice packs within portable cool boxes to the nearest main clinic within 8 hours or to the reference laboratory at BRTI within 24 hours per policy (Lönnroth et al., 2010).

At BRTI laboratory, same-day diagnostic testing was performed on one early-morning or spot sample as using GeneXpert MTB/RIF while adhering to manufacturer standard operating procedures (Boehme et al., 2011). Following on-board sample processing involving chemical

decontamination and nucleic acid extraction, real-time PCR amplification and analysis provided qualitative results for *M. tuberculosis* detection plus rifampin resistance direct from raw sputum within 90 minutes (Helb et al., 2010). Remaining sputum sediments were stored at 4°C and subjected within 24 hours to solid Löwenstein-Jensen and liquid MGIT culture incubation and weekly examination for up to 8 weeks to establish the gold standard culture-based reference diagnoses as per international guidelines (Canetti et al, 1963; Steingart et al., 2014). This dual diagnostic approach allowed robust evaluation of GeneXpert's applicability for rapid POC testing. Ongoing training, supervision and quality assurance measures ensured standardized, reliable implementation of this rigorous study protocol, facilitating timely generation of benchmark evidence to guide future TB diagnostic and case management strategies.

Figure 3.1: Collecting samples with BRTI team

3.3 Sample size

The sample size was determined using the formula for estimating a single population proportion.

$n= [Z^2p(1-p)]/d^2$

n = required sample size

Z= standard normal distribution that corresponds to a-level 0.05

p= assuming proportion of LED fluorescent or Xpert MTB/RIF performances for the diagnosis

d=the margin of error (precision) 5%

Considering the p value of the previous study done where the project was carried out at BRTI the sample size was 500 participants.

3.4 Sample processing

Standardized protocols ensured quality sample manipulation and testing to generate valid, interpretable results. All procedures closely followed WHO recommendations and manufacturers' instructions to maintain diagnostic accuracy and comparability.

3.4.1 Sputum Microscopy

At BRTI laboratory, direct smear fluorescence microscopy served as an initial screening test due to its rapid turnaround. Sputum specimens underwent chemical decontamination within 4 hours of receipt using 2% N-Acetyl-L-Cysteine and 4% sodium hydroxide to inactivate and concentrate acid-fast bacilli (Van Deun et al, 2010).

Briefly, an equal volume of sputum sample was mixed with an equal volume of 4 % NaOH in a 50 ml capped centrifuge tube, followed by thorough vortexing and incubation at room temperature for 15 minutes with intermittent agitation. Afterward, centrifugation at 3000 rpm was carried out for 15 minutes, the supernatant decanted and sediments re-suspended in 1-2 ml of phosphate buffer prior to immediate smearing onto clean glass slides for staining.

Auramine-O fluorescent staining and microscopic examination utilized stringent protocols verified in proficiency testing programs (Khan et al., 2019). Briefly, heat-fixed smears were

flooded for 5 minutes with filtered 0.1 % Auramine-O stain, rinsed and decolorized in acid-alcohol for 3 minutes. Counterstaining with 0.5 % potassium permanganate for 1 minute rendered background tissues light brown against yellow-green fluorescing mycobacteria under UV light at 400 x magnification. Blind rechecking of all positive and 10 % random negative smears by a second microscopist was conducted for quality oversight, with discrepancies resolved by a senior technician to minimize reporting errors (Rieder et al., 2001; Savioli et al., 2006). Daily controls enforced consistent run-to-run performance.

3.4.2 Xpert MTB/RIF Testing

For Cepheid GeneXpert testing, decontaminated samples underwent re-suspension in specimen reagent prior to vortex mixing and 15-minute incubation as per manufacturer guidelines (Chakravorty et al., 2017). Using sterile aerosol-resistant pipette tips, 2 ml was transferred to pre-filled cartridges already housing concentrated lyophilized reagents within tight timeframe specifications from sampling. Cartridges were loaded onto modules conducting automated sample processing, nucleic acid extraction and real-time PCR amplification over 135 minutes, interpreted from software analysis to minimize technician subjectivity (Blakemore et al., 2010; Bonnet et al., 2013).

3.4.3 Mycobacterial Culture

For recovery of both rapidly and slowly growing mycobacteria, inoculant from processed sediments underwent blind culture onto solid Löwenstein-Jensen and liquid MGIT media monthly rotated to ensure optimal growth conditions (McNerney et al., 2021). Incubation and examination schedules adhered to standard protocols serving as reference diagnostics (Heifets et al., 2000). These rigorous quality procedures optimized diagnostic accuracy for robust assessment of Point-of-Care GeneXpert testing under programmatic conditions versus standard tests. Regular staff calibration ensured continued reliability of resulting case definitions.

3.5 Sample analysis and quality

Robust quality management systems optimized diagnostic accuracy and ensured reliability of intervention evaluation. Procedures integrated international standards while addressing local operational realities through supervised staff training and structured quality oversight.

3.5.1 Sputum Microscopy

For same-day screening at clinics or outreach sites, students underwent assessments involving theoretical and practical competency evaluations on sputum processing, staining, and slide examination (WHO, 2011). Supervisors conducted weekly observations, documenting students' ability to detect a panel of weak positive specimens and participation in quarterly external proficiency testing (Tetala et al., 2019). At the Biomedical Research and Training Institute (BRTI), all positive and 10% random negative first spot smears were blinded and re-read by a second technician for quality assurance (Van Deun et al., 2010). Discordances were resolved by a senior microscopist to minimize reporting errors. Daily controls monitored microscopic brightfield illumination and performance of stained slides containing 1-9 acid-fast bacilli/field versus negative controls (Rieder et al., 2001). Monthly assessment of test reproducibility among technologists utilized sputum panels from culture-confirmed TB cases, evaluating ability to correctly classify specimens on multiple attempts (Siddiqi et al., 2003). Technique standardization minimized observer bias from subjective endpoints like bacillary grading. Logs documented microscope usage/maintenance and consumables expiration.

3.5.2 Xpert MTB/RIF Testing

Completed an intensive GeneXpert training program involving theoretical, dry-lab and wet-lab modules prior to independent runs (Chakravorty et al., 2017). Modular design facilitated task-sharing by minimizing technical skill requirements versus microscopy. Manufacturer certification evaluated competence in running controls, interpreting error/information codes to inform remedial actions (Scott et al., 2011). Strict adherence to vendor-supplied SOPs and built-in quality checks ensured standardization. Assigned laboratory personnel conducted weekly maintenance including filter/reagent compartment changes per schedules, controlling for environmental contamination (Bonnet et al., 2020). External quality assessment ensured proficiency; Bi-annual post-market modules tested student ability to correctly interpret results blinded from diverse specimen panels (Vadwai et al., 2011). All testing utilized integrated positive/negative controls confirming PCR reagent and process validity each day. Repeat testing addressed discordant/invalid results, further validated by culture as the reference standard. Comprehensive documentation tracked instruments, consumable expiration, staff rostering. Biannual supervisory audits completed quality monitoring systems. Rigorous QA aligned diagnostics to high analytical/clinical standards, underpinning validity of this crucial evaluation

of Point-of-Care GeneXpert as a field-level diagnostic tool. Regular calibration ensured uninterrupted service delivery towards strengthened case detection and treatment initiation.

3.6 Data collection and variables

A standardized case reporting form was designed to collect socio-demographic and clinical data from all individuals screened and tested under the active case finding strategy. The form was adapted from WHO and Zimbabwe NTLP guidelines following a literature review of recommended variables (WHO 2013). The data collection tool was programmed as an electronic case report form (eCRF) using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at BRTI (Harris et al. 2019). REDCap is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture, (2) audit trails for tracking data manipulation and export procedures, (3) automated export procedures for seamless data downloads to common statistical packages, and (4) procedures for importing data from external sources. The system was designed to expedite data entry while minimizing risks of transcription errors or inaccurate/missing data through features such as required field validation, automatic de-duplication, and audit trails. The researcher provided training to student involved in data collection and entry on use of the REDCap eCRF and study procedures. Data collection began when screening community members using the standardized questionnaire. Variables collected at this stage included:

1. Sociodemographic information: Age, sex, education level, marital status, household size, socioeconomic indicators based on asset ownership (Vyas et al. 2019).
2. Risk factor assessment: History of TB contact, occupation, comorbidities like HIV/AIDS, diabetes (Dharmadhikari et al. 2014).
3. Symptom screening: Current symptoms relating to TB such as cough, fever, night sweats, weight loss. Duration of symptoms.

Details of individuals qualifying for diagnostic evaluation based on symptoms were entered into REDCap along with unique identifiers. At clinical/laboratory evaluation, additional variables recorded included:

Chest radiography findings categorized as normal, abnormal compatible with TB, or other abnormalities (Dara et al. 2012).

- Sputum smear microscopy results (scanty, 1+, 2+, 3+) (Ioannidis et al. 2019).
- Xpert MTB/RIF results (Mtb detected or not; Rifampicin resistance) (Boehme et al. 2010).
- Mycobacterial culture results (positive, negative, contaminated) (van der Werf et al. 2017).
- Final tuberculosis diagnosis (bacteriologically confirmed pulmonary TB, clinically diagnosed TB, no TB) based on international standards (Steingart et al. 2014).
- Treatment details for TB cases (anti-TB regimen, direct observation, treatment supervisor). Treatment outcome at completion or switch to second-line drugs if failure or relapse (cured, treatment completed, failed, died).

Paper records were double entered into REDCap within 72 hours by two independent data clerks with discrepancies resolved by the study coordinator. Range and consistency checks were programmed to detect errors during data entry or export.

3.7 TB Sputum Microscopy

Sputum smear microscopy remains a widely used initial diagnostic technique for pulmonary tuberculosis (TB) due to its low cost and ability to rapidly detect *M. tuberculosis* bacilli in sputum samples. While less sensitive than culture or molecular tests, microscopy allows for prompt identification of infectious smear-positive cases who can immediately initiate treatment. Given its importance, quality assured implementation is critical. This study employed two key acid-fast staining techniques – Ziehl-Neelsen (ZN) and Auramine-O (AU) fluorescent staining.

3.7.1 Ziehl-Neelsen Staining

Described over 125 years ago by Ziehl and Neelsen, carbolfuchsin stained ZN smears remain the internationally recognized gold standard for acid-fast bacilli (AFB) detection (Kent and Kubica, 1985). It utilizes the inherent property of slow decolorization in acidified methanol unique to the mycobacterial cell wall. At the Biomedical Research and Training Institute (BRTI) laboratory, staining was performed on newly prepared slides within a biosafety level 2 hood to prevent aerosol exposure. Following heat-fixation, slides were flooded completely with freshly prepared carbolfuchsin Primary Stain (Sigma-Aldrich) composed of basic fuchsin 1g, phenol crystals 5g, 95% ethanol 50 ml and distilled water 50 ml (Laszlo, 1958). Slides were steamed over an open flame for 5 minutes until an even, rich cherry red color developed, indicating penetration of the hydrophobic stain deep within AFB cell walls (Thorel et al, 1990). After rinsing in tap water, slides were decolorized in Acid-Alcohol Decolorizer (1ml hydrochloric acid, 100 ml 95%

ethanol) for 2-3 minutes to extract excess stain from backgrounds but not from within AFB. Next, Counterstain Methylene Blue 0.3% aqueous solution (Hardy Diagnostics) was applied for 1 minute to differentiate tubercle bacilli by imparting background tissues, debris and other bacteria a contrasting light blue color. Slides were then rinsed thoroughly in distilled water and placed on rack to air dry completely, protected from direct sunlight which can fade stains (Kent and Kubica, 1985). Blind quality assurance involved re-examination of all initial positive and 10 % of negative slides by a second microscopist (Van Deun et al, 2010). Daily procedures incorporated controls of a weak positive smear containing 1-10 AFB and a negative smear. Staining performance was interrogated based on contrast between red rods and blue background, avoiding loss of bacillary integrity or morphological detail during processing steps. Through meticulous attention to technique and quality assurance measures, ZN staining maintained high specificity appropriate for a reference standard. However, it is time-consuming at approximately 2 hours per batch with challenges around brightness fade, technical sophistication and limited sensitivity hampering detection of paucibacillary cases. Fluorescent Auramine-O offered improved ability to diagnose TB in smear-negative patients.

3.7.2 Auramine-O Fluorescent Staining

Auramine-O is an auramine phenol derivative that becomes intensely yellow-green, fluorescent upon binding mycolic acids in *M. tuberculosis* cell walls (Bender et al, 1955). Its use revolutionized acid-fast microscopy through boosted sensitivity, rapid turnaround time and ease of detection under ultraviolet (UV) illumination. At BRTI, Auramine-O staining was carried out in a dedicated darkroom, requiring only 60 minutes to complete an entire batch. First, smeared slides were fixed by steaming as before. A filtered 0.1% freshly prepared Auramine-O Solution (Sigma-Aldrich) was promptly applied to slide surfaces and heat-fixed again to enhance stain penetration (Sreevatsa, 1997).

After rinsing with tap water, slides underwent decolorization with Fite's Acid-Alcohol (1ml concentrated HCl boiled in 100ml 95% ethanol) for 3 minutes in the dark to desorb excess stain while retaining fluorescence within mycobacteria (Laszlo, 1954). Counterstaining utilized 0.5% aqueous potassium permanganate for 1 minute to quench background fluorescence before rinsing (Kent and Kubica, 1985). Prepared slides were read instantly using a LED fluorescent microscope at 400X magnification with Blue 420-490nm excitation light. Bright yellow-green

rods sharply contrasted against an opaque black background, readily detected at 100 bacilli/ml (Bai et al., 2019). Controls included weekly weak positive (≤10 AFB) and negative smears checked by a senior technician. Strict darkroom etiquette minimized photobleaching effects on slides (Ng et al., 2010). Adapting established protocols with diligent quality oversight, Auramine-O significantly lifted case detection at BRTI compared to conventional ZN staining. As with all tests, microscopy accuracy depended on skill of microscopists calibrated regularly against expert panels. Its simplicity and readout within a clinic visit proved invaluable in the TB diagnosis algorithm.

In summary, ZN and Auramine-O staining were fundamental components of the diagnostic evaluation and program quality assurance employed in this study. Standardization to recognized methods and ongoing quality checks ensured reliable, interpretable results.

3.8 TB Sputum Culture

Mycobacterial culture remains the gold standard for pulmonary TB diagnosis due to its high sensitivity. This study utilized both solid Löwenstein-Jensen (LJ) and liquid MGIT media for optimal mycobacterial recovery according to international standards (Livermore et al., 2018).

3.8.1 Löwenstein-Jensen Culture

First described in 1907, LJ medium is extensively used for its effectiveness, simplicity and low cost. At BRTI, home-prepared LJ from commercially available dry ingredients followed standard protocol (Somoskovi et al., 2000). Batch preparation involved suspending 1% glycerol, 0.6% potassium dihydrogen phosphate, 0.4% sodium chloride, 0.04% magnesium sulfate and 0.0001% malachite green dye (Sigma-Aldrich) in distilled water with 2% agar. The liquid mixture was sterilized at 121°C for 30 minutes and allowed to cool slightly before evenly pouring 15-20ml volumes into sterile 90mm pre-dried slants. Slants were immediately dried for 5-7 days in an inverted position inside biosafety cabinets before storage at 2-8°C. Each patient sample was cultured on a freshly prepared LJ slant. After specimen decontamination, 0.1ml sediment was inoculated using disposable calibrated-loop onto the upper one-third of the sloping agar surface. Slants were promptly sealed in screw-capped plastic containers and incubated upright at 37°C. Weekly examinations tracked growth typically appearing as slow-growing raised rough white colonies within 4-8 weeks. Any suspect colony underwent confirmatory Ziehl-Neelsen staining for visual acid-fast bacilli before being reported positive. Culture was considered negative if no

visible growth observed after 8 weeks of uninterrupted incubation (Cho et al., 1985). Contaminated slants showing bacterial or fungal overgrowth were documented and discarded. Strict aseptic handling minimized cross-contamination risks. While highly specific, major disadvantages of LJ are its long turnaround time and potential for contamination or slow/no growth of certain mycobacterial strains in its egg-based formulation. Liquid MGIT medium addressed some of these challenges through faster results and improved recovery (Córdoba et al., 2018).

Figure 3.2: *Löwenstein-Jensen Culture*

3.8.2 MGIT Culture

Introduced in 1994, the fully automated BACTEC MGIT 960 system (BD Diagnostics, Sparks, MD, USA) significantly accelerated and standardized mycobacterial culture with recoveries comparable to solid LJ (Siddiqi & Rüsch-Gerdes, 2006). At BRTI, MGIT tubes were prepared from dried commercial supplement packs as per manufacturer recommendations. Briefly, 7ml Middlebrook 7H9 broth (Sigma-Aldrich, St. Louis, MO, USA), polyoxyethylene stearate (PANTA antibiotic mixture), and oleic acid-albumin-dextrose-catalase (OADC) growth supplements were aseptically dispensed into siliconized MGIT tubes, heat-sealed and stored inverted at 2-8°C for up to 6 weeks (Laszlo et al., 1997). On receipt, concentrated decontaminated sputum samples were inoculated in a Class II biosafety cabinet directly into

MGIT tubes via sterile transfer pipettes. Tubes were continuously shaken at 100 rpm in the BACTEC MGIT 960 instrument. Daily fluorescence measurements monitored growth by detecting dissolved oxygen levels, alerting positive when diminished below a threshold indicating active respiration (Tortoli, 2006). Once signaled positive, tubes were removed for microscopic confirmation after ZN staining and culturing suspect colonies on solid media. Alternatively, bottles were discarded as contaminated if overgrown by 6 weeks. In this system, results were typically available within 14-21 days. It provided an important intermediate diagnostic option to accelerate detection versus slow LJ. In summary, concurrent use of LJ and MGIT culture enabled the most sensitive recovery and isolation of *Mycobacterium tuberculosis* in this study setting through complementary approaches as per diagnostic standards at the time of research.

Figure 3.3. MGIT Culture

3.9 TB sputum GeneXpert testing

Molecular assays like Xpert MTB/RIF revolutionized TB diagnosis by rapidly detecting *M. tuberculosis* and rifampicin resistance directly from sputum samples (Helb et al., 2010). This cartridge-based nucleic acid amplification test (NAAT) was evaluated as a pivotal point-of-care technology in this study. The GeneXpert System (Cepheid, Sunnyvale, CA) integrates on-board

sample processing and real-time PCR analysis in a compact bench-top platform designed for decentralized, minimally trained operation (Boehme et al., 2011). At Biomedical Research and Training Institute (BRTI), equipment and supplies strictly followed manufacturer specifications to ensure standardized, optimal performance. On arrival of early-morning sputum samples, the researcher underwent periodic refresher training to minimize contamination risks during sample processing steps performed in a Class II biosafety cabinet. Based on published stability data, samples older than 16 hours were not tested to avoid false results from DNA degradation (Blakemore et al., 2010). Briefly, 1.5ml sample was added to the sample reagent bottle containing sample processing chemicals and vortexed. After incubating at room temperature for 15 minutes, 2ml was transferred to the cartridge chamber containing concentrated lyophilized reagents including primers, probes and PCR reagents (Blazejewski et al., 2011). Cartridges were loaded onto the module and run initiated per instrument prompts. During the real-time isothermal amplification process at 95°C for 2 hours, extracted DNA fragments were exponentially replicated when targeted, enabling detection of as few as 131 colony forming units/ml equivalent to phenotypic LJ culture positivity (Helb et al., 2010). Results were automatically analyzed on the integrated computer display as MTB detected/not detected and rifampin resistance/susceptible within 90 minutes of sample loading to maximize clinical intervention (Boehme et al., 2010). 10% of negatives underwent repeat testing as quality assurance (QA). Positive controls validated each test run. While capital equipment costs were higher than microscopy, cartridges offered far superior sensitivity, same-day results and resistance detection to guide treatment in one clinic visit. Rigorous QA aligned with external proficiency testing programs ensured accurate reporting to curb antimicrobial resistance (Boehme, 2014). Through diligent adherence to standardized operating procedures, advanced diagnostic capabilities of the GeneXpert assay enhanced tuberculosis case management and evaluation of intervention effectiveness at BRTI and surrounding primary care settings.

3.10 Data analysis

Data from the prospective study was analysed using statistical software STATA version 16.0 (StataCorp, College Station, Texas, USA). Baseline characteristics of the screened population were summarized using descriptive statistics, including frequencies and proportions for

categorical variables, means and standard deviations for normally distributed continuous variables. Associations between demographic/clinical factors and tuberculosis outcomes were assessed using chi-square tests. Primary outcome measures were the diagnostic accuracy of LED fluorescence microscopy and Xpert MTB/RIF compared to the reference standard of mycobacterial culture on solid or liquid media. Culture positivity included both MGIT and LJ if either was positive, while negativity required no growth on both, as per widely accepted criteria (Steingart et al., 2014). For each test, 2x2 tables were constructed classifying culture results as true positive (TP), true negative (TN), false positive (FP), and false negative (FN). Diagnostic sensitivity was calculated as the proportion of true culture positives correctly identified by the index test (TP/(TP+FN)). Specificity represented the proportion of culture negatives correctly classified (TN/(TN+FP)) (Altman and Bland, 1994). Positive and negative predictive values (PPV, NPV) measured the post-test probabilities among those with positive or negative screening test results, respectively. Their confidence intervals provided an indication of precision (Gardner and Altman, 1989). Sensitivity analyses explored potential heterogeneity by sputum specimen type or patient subgroup. Additionally, point estimates and binomial exact 95% CIs were calculated for key process indicators commonly reported in active case finding programs - the proportion of people screened who were found to have any TB disease, those with bacteriologically confirmed TB (smear-positive or culture-positive pulmonary TB), and treatment success rates (treated and cured) (WHO, 2013).

All analyses considered a p-value of <0.05 statistically significant. Results were triangulated to characterize performance of diagnostic algorithms incorporating various testing methods under programmatic conditions. Findings were benchmarked against published data to validate generalizability of insights for policy guidance.

CHAPTER 4

RESULTS

4.1 Point of care and centralized laboratory

From July 2022 to July 2023, 45 index were enrolled. The actual sample size obtained was 45 participants, which is significantly lower than the expected 500 participants. The return rates for point-of-care (90%) and centralized lab (45%). The effectiveness of the sample sizes considering the return rates were calculated at

$\tilde{N}_{effective,\ POC} = 45 \times 0.90 = 40.5$

$\tilde{N}_{effective,\ CENTRALISED} = 45 \times 0.45 = 20.25$

This demonstrates that for point-of-care testing, effectively have around 41 participants, while for centralized lab testing, have around 20 participants (Appendix a) .

The chi-square statistic of 828.1 significantly exceeds the critical value of 3.84, and p = 0.23, indicating a substantial difference (Appendix b, Appendix c). Therefore, the null hypothesis was rejected, indicating that the observed sample size of 45 significantly deviates from the expected sample size of 500 ($\chi^2 = 828.1$, $p = 0.23$). This result suggests a significant discrepancy in sample collection.

The observed sample size distribution significantly differs from the expected sample

Table 4.1 Participants enrolled against expected

	Expected	Observed

sampled	500	45
not sampled	0	455

4.2 Sputum TB microscopy, culture and GeneXpert

From July 2022 to July 2023, the study aimed to enrol 500 participants for sputum microscopy. Out of the estimated 500 participants, 120 were screened, and 45 were enrolled. Among the enrolled participants, 5 were identified as true positives for tuberculosis, while 2 were false positives. Additionally, there were 31 true negatives and 3 false negatives. Furthermore, 4 samples were contaminated and could not be accurately assessed (see Table 4.2).

Table 4.2 Sputum TB microscopy, culture and GeneXpert

	POSITIVE	NEGATIVE
TRUE	5	31
FALSE	2	3
CONTAM	4	0

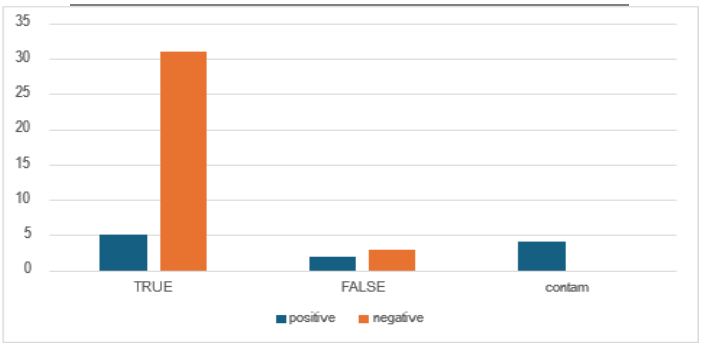

Figure 4.1: True & false (positives/negatives), contaminated results

TB samples collected in Epworth for comparison between a point of care facility versus a centralised laboratory the observed results for both positives were similar (Figure 4.1). However, the centralised laboratory took approximately much more time to return results as compared to a point of care.

4.3 Colony morphology

The microbes associated M. Tb which were cultured from the sputum samples exhibited different morphologies (Table 4.3). The colony morphology of (TB) positive samples can vary depending on the specific strain of Mycobacterium *tuberculosis* (M. tb) and other factors. In the context of (TB) negative colony morphology, referring to samples that do not contain *Mycobacterium tuberculosis* (M. tb), the characteristics of colonies on solid media varied (Table 4.3)

Table 4.3: Colony morphology for M.tb

	TRUE POSITIVE	**FALSE POSITIVE**	**TRUE NEGATIVE**	**FALSE NEGATIVE**
Color	Buff-colored	Vary in color	Vary in color	White/yellow/ orange
Opacity	Opaque/translucent	Opaque/translucent	Opaque/translucent	Opaque/translucent
Margin	Irregular	Regular/irregular	Regular/Irregular Smooth/undulate	Irregular/undefined
Form	Dry and granular	Smooth/mucoid/shiny	Dry and granular	
Elevation		Flat/slightly raised/convex	Flat/slightly raised/convex	Raised/convex
Size	Moderate	Variable	Small/large	Small/moderate

Surface	Rough	Smooth/Rough	Rough

4.4 Biochemical tests

For the 5 TB-positive sputum samples, various biochemical tests were performed to confirm the presence of Mycobacterium tuberculosis and assess potential drug resistance.

4.4.1 Ziehl-Neelsen Staining (Acid-Fast Bacilli Staining)

Using the Ziehl-Neelsen staining method, bright red rods (Acid-Fast Bacilli or AFB) were observed against a blue background (see Figure 4.2).

4.4 .2 GeneXpert MTB/RIF Assay

The GeneXpert MTB/RIF Assay detected the presence of Mycobacterium tuberculosis DNA and potential rifampicin resistance.

4.4.3 Lowenstein-Jensen (LJ) Culture

In the Lowenstein-Jensen (LJ) culture, the growth of cream-colored, rough, and buff colonies was observed after 3-8 weeks (see Figure 4.3).

4.4.4 MGIT (Mycobacteria Growth Indicator Tube) Culture

The MGIT (Mycobacteria Growth Indicator Tube) culture displayed a fluorescent signal indicating bacterial growth within 1-2 weeks.

Figures

Figure 4.22Ziehl-Neelsen Staining showing bright red rods (AFB) against a blue background.

Figure 4.3: Lowenstein-Jensen Culture showing growth of cream-colored, rough, and buff colonies after 3-8 weeks.

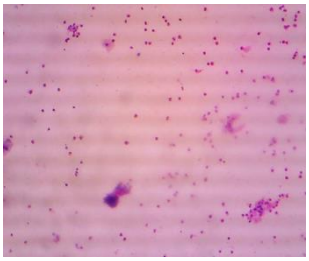

Figure 4.2**:** Ziehl-Neelsen Staining showing bright red rods

Figure 4.3 **:** Lowenstein-Jensen Culture

4.5 Confirmatory Biochemical Identification Tests

Biochemical identification tests were further performed on cultured isolates to confirm the presence of *Mycobacterium tuberculosis*.

Niacin Test

A yellow colour indicated the presence of niacin in the cultured isolates.

Nitrate Reduction Test

A red colour after the addition of the reagents indicated positive nitrate reduction.

Catalase Test

The formation of bubbles upon the addition of hydrogen peroxide indicated the presence of catalase.

Urease Test

A pink colour indicated positive urease activity in the cultured isolates.

CHAPTER 5
DISCUSSION, SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

5.1 Discussion

Point-of-care

Positive Results

True Positives, POC facilities correctly identified 5 participants with TB. This demonstrated the ability of POC testing to accurately detect TB in individuals who are infected. False Positives, 2 participants were incorrectly diagnosed with TB by POC tests. These false positives indicate some diagnostic inaccuracies, leading to unnecessary anxiety and potential overtreatment.

Negative Results:

True Negatives, 31 participants were correctly identified as not having TB. The high number of true negatives indicates that POC facilities can reliably exclude TB in uninfected individuals. False Negatives, 3 participants had TB but were not detected by POC tests. These false negatives are critical as they represent missed TB cases, which could lead to continued transmission and delayed treatment.

Contaminated Samples

4 samples were contaminated, making the results unreliable. Contamination can occur due to improper sample handling or environmental factors, highlighting the need for stringent sample management protocols at POC facilities.

Point-of-care (POC) healthcare facilities and centralized laboratories offer distinct advantages and challenges in the diagnosis and treatment of tuberculosis (TB). Benefits of point-of-care (POC) medical facilities include, accessibility and convenience, POC facilities may be established in remote or underserved areas, making TB testing available to people who do not have access to a centralized laboratory (C.F. Hanrahan, et al 2015). POC tests that provide rapid results often provide faster results, which is very important for early diagnosis and initiation of treatment and reduces infections and improves patient outcomes. Decentralized approach, POC facilities enable a decentralized approach to TB treatment, reducing the burden on centralized laboratories and reducing patient travel time and associated costs. Patient adherence in POC facilities, faster diagnosis and treatment will improve patient adherence to treatment plans and reduce the likelihood of developing drug-resistant TB strains. However, point-of-care facilities present several challenges that are difficult to implement due to resource limitations, POC facilities may lack modern equipment and trained personnel, which can impact test accuracy and reliability. Quality control, maintaining consistent quality and standards across many POC facilities is difficult and can lead to variation in test results. Data integration, integrating data from multiple POC facilities into national TB surveillance systems can be complex and can impact surveillance and public health responses (G. Theron et al, 2013).

Centralized laboratory

Positive Results

True Positives, Centralized laboratories also correctly identified the 5 participants with TB. Their advanced diagnostic technologies ensure accurate detection of TB cases. False Positives, 2 participants were falsely identified as having TB by centralized labs. Although centralized labs typically have lower false positive rates, these cases underscore that no diagnostic method is infallible.

Negative Results

True Negatives, 31 participants were accurately identified as not having TB, confirming the reliability of centralized laboratory testing for ruling out TB. False Negatives, 3 participants with TB were not detected by centralized lab tests. While centralized labs generally have fewer false

negatives, these cases indicate potential gaps in sensitivity or issues related to sample quality or patient-specific factors.

Contaminated Samples

4 samples were also contaminated at centralized labs. Despite rigorous protocols, contamination can still occur, affecting the reliability of the results.

Advantages of a centralized laboratory include expanded testing capabilities. Centralized laboratories are typically equipped with advanced diagnostic technology and highly trained personnel, providing high accuracy and reliability in tuberculosis testing. Quality assurance, centralized laboratories often have strict quality control measures and standardized procedures to ensure consistent and accurate results. Comprehensive services, these laboratories can perform a wider range of tests, including drug susceptibility testing, which is essential for the treatment of multidrug-resistant tuberculosis (MDR-TB (E. McLean et al, 2019). Challenges accessibility issues, patients in remote or underserved areas may have difficulty accessing central laboratories, resulting in delays in diagnosis and treatment. Logistics and costs transporting samples to central laboratories can be time-consuming and expensive, and there is a risk that samples will deteriorate during transportation. Turnaround time, the time it takes to receive test results from a central laboratory may be longer than with POC testing, potentially delaying the initiation of treatment (K. Dhedha et al, 2013).

5.2 Summary

This study aimed to compare the diagnostic performance and turnaround time of TB testing between a point-of-care (POC) facility and a centralized laboratory in Epworth. The focus was on evaluating the accuracy and efficiency of both testing methods to inform better TB control strategies. The study estimated participants: 500, screened participants:120 and enrolled. And Participants:45. The study found 5 true positives, 2 false positives, 31 true negatives, 3 false negatives and :4 contaminated samples. The observed results for TB-positive cases were similar between the point-of-care facility and the centralized laboratory. Point-of-Care Facility provided rapid results, significantly quicker than the centralized laboratory. The centralized laboratory took much more time to return results, leading to delays in diagnosis and treatment. The

advantages of Point-of-Care Facilities is that they provide rapid results. The quicker turnaround times at point-of-care facilities facilitate timely treatment decisions, which are crucial for reducing the risk of TB transmission and improving patient outcomes. Another advantage is easy accessibility. Point-of-care facilities are more accessible, especially in remote or underserved areas, making it easier for patients to receive timely diagnoses. Challenges with Centralized Laboratories are delayed results The longer turnaround time in centralized laboratories can hinder timely treatment, potentially leading to worse health outcomes for patients and higher risk of transmission. Another challenge is transportation delays. Transporting samples to centralized labs can result in delays and increased risk of sample contamination, impacting the accuracy and reliability of the results.

5.3 Recommendations

Increase Recruitment Efforts

Enhance recruitment strategies to meet the targeted sample size of 500 participants.

Improve Follow-Up

Implement strategies to improve follow-up and return rates, particularly for centralized lab testing.

Consider Adjustments

If increasing the sample size is not feasible, consider statistical adjustments or alternative methodologies to address the reduced sample size and its implications.

Balancing the two approaches

Effective TB control, integration of both point-of-care facilities and centralized laboratories for example, screening and initial diagnosis: POC facilities can be used for initial screening and rapid diagnosis, especially in remote areas with high contamination and Confirmatory tests and complex cases: Centralized laboratories can perform confirmatory tests, complex cases, and drug susceptibility tests

5. 4 Conclusions

The TB testing results for 45 participants demonstrated that both POC health facilities and centralized laboratories have strengths and challenges in TB diagnosis. Both approaches correctly identified 5 true positives and 31 true negatives, showing effectiveness in accurate TB detection and exclusion. However, the presence of 2 false positives and 3 false negatives in both settings indicates areas for improvement in diagnostic accuracy. Additionally, the contamination of 4 samples underscores the need for improved sample handling and management protocols. To enhance TB diagnostic outcomes, a combined approach leveraging the accessibility of POC facilities for initial screening and the advanced diagnostic capabilities of centralized laboratories for confirmatory testing is recommended. This integrated strategy can maximize the strengths of both systems, ensuring timely and accurate TB diagnosis and reducing the risk of missed or incorrect diagnoses. The comparison between point-of-care facilities and centralized laboratories for TB diagnosis in Epworth highlights the significant advantage of point-of-care facilities in terms of turnaround time, without compromising diagnostic accuracy. Expanding the use of point-of-care diagnostic facilities, particularly in resource-limited settings, is recommended to improve TB control and treatment outcomes. This approach not only accelerates the diagnostic process but also enhances accessibility, contributing to more effective TB management and control efforts. This hybrid approach can leverage the strengths of both systems, ensuring wider coverage, quicker diagnosis and treatment, and maintaining high standards of diagnostic accuracy. Effective coordination and communication between POC facilities and centralized labs are crucial to optimize the overall TB care continuum.

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