Evaluating the Performance of Xpert MTB/RIF and Its impact on Patient Level Outcomes among Individuals Attending Routine Health Facilities for HIV Care and Treatment in Botswana

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Declaration

This thesis submitted in the integrated format, approved by the Faculty of Health Sciences, of published work with an encompassing introduction and conclusion.

I, Tefera Agizew, declare that this thesis is my original work. It has been duly acknowledged where there has been a contribution from other people. It is being submitted for the degree of Doctor of Philosophy in Public Health in the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this or any other University.

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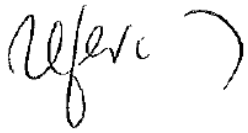

Date: December 16, 2019

Dedication

For my dearest wife Senait Tekola Gebremedhin, and my dearest sons – Abel Belachew and Bereket Belachew and daughters – Hulumelkam Belachew and Wubet Belachew.

Presentations arising from this PhD

1. **Agizew** T, Auld A, Date A, Madidimalo T, Moalosi G, Ndwapi N, e al. Impact and operational challenges of use of Xpert MTB/RIF on TB case finding in PLHIV in Botswana. 43rd Union World Conference against TB and Lung Disease, Kuala Lumpur, Malaysia, Symposium presentation by invitation, November 15, 2012.
2. **Agizew T**, Basotli J, Alexander H, Boyd R, Auld A, Date A, et al. Higher-than-expected non-tuberculous mycobacteria disease prevalence in HIV setting in Botswana: implications for Xpert MTB/RIF diagnostic algorithms. Oral presentation 45th Union World Conference against TB and Lung Disease, Barcelona, Spain. Abstract No. OAP-212-30. October 30, 2014.
3. **Agizew T**, Nyirenda S, Mathoma A, Mathebula U, Date A, Kgwaadira B, et al. Comparison of Pre- and Post-Xpert Tuberculosis Treatment Outcomes among People living with HIV in Botswana. Poster Discussion Session. 46th Union World Conference against TB and Lung Disease Cape Town, South Africa. Abstract No. PC-1124-06. December 6, 2015.
4. Basotli J, **Agizew T**, Radisowa K, Rey Z, Tamuhla T, Alexander H, et al. Identification and characterization of non-tuberculous mycobacterial infection among people living with HIV presenting with TB symptoms in Botswana. ePoster Discussion Session. 46th Union World Conference against TB and Lung Disease Cape Town, South Africa. Abstract No. EP-179-05. December 6, 2015.
5. Zimba O, Tsaone T, Basotli J, Letsibogo G, Pilara K, Mathebula U, Mathoma A, Serumola C, Alexander H, Boyd R, Tran T, Finlay A, **Agizew T**. Effect of sputum quality and volume on the yield of bacteriologically confirmed TB by Xpert and smear microscopy among people living with HIV in Botswana. TB2016 international conference, Durban, South Africa, Abstract No. A-816-0007-00136, July 17, 2016.
6. **Agizew T**, Finlay A, Boyd R. Systematic review and meta-analysis of clinical outcomes of drug-sensitive tuberculosis diagnosed by Xpert MTB/RIF and smear microscopy. Oral presentation 47th Union World Conference against TB and Lung Disease, Liverpool, UK. Abstract No. OA-404-28. October 28, 2016.

7. **Agizew T**, Boyd R, Mathebula U, Mathoma A, Basotli J, Serumola C, et al. Treatment outcomes among people living with HIV with non-tuberculosis mycobacteria versus mycobacterium tuberculosis in Botswana. Oral presentation 48th Union World Conference against TB and Lung Disease, Guadalajara, Mexico. Abstract No. OA-121-12. October 12, 2017.

Other presentations complementary to the PhD

8. Finlay A, **Agizew T**, Date A, Boyd R, Pals S, Alexander H, et al. Tuberculosis prevalence and incidence among people living with HIV attending HIV care and treatment clinics in Botswana, August 2012-October 2014. Oral presentation 47th Union World Conference against TB and Lung Disease, Liverpool, UK. Abstract No. OA-372-28. October 28, 2016.
9. Mathebula U, Emerson C, **Agizew T**, Boyd B, Mathoma A, Pals S, Auld A, Finlay A. Improving sputum collection to increase tuberculosis case finding among HIV- positive persons enrolling in HIV care and treatment clinics in Botswana, 2012-2014. Oral presentation 48th Union World Conference against TB and Lung Disease, Guadalajara, Mexico. Abstract No. OA-118-12. October 12, 2017.
10. **Agizew T**, Letebele M, Auld AF, Pals S, Mathebula U, Pono P, et al. Yield of repeated tuberculosis screening among HIV patients: Implications for tuberculosis preventive therapy. Oral presentation. 49th Union World Conference against TB and Lung Disease, Hague, the Netherlands, Abstract No. A-0996-0013-02394, October 25, 2018.

    Auld A, **Agizew T**, Mathoma A, Boyd B, Date A, Pals P, et al. Effect of TB screening and retention interventions on early art mortality in Botswana. Oral abstract 31, 25th Conference on Retroviruses and Opportunistic Infections, Boston, Massachusetts, March 4-7 2018.

Publications arising from this PhD

1. **Agizew T**, Boyd R, Ndwapi N, Auld A, Basotli J, Nyirenda S, et al. Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic, stepped-wedge trial in Botswana. PLoS One 2017 17;12(8):e0183237.
2. **Agizew** T, Chihota V, Nyirenda S, Tedla Z, Auld AF, Mathebula U, et al. Tuberculosis treatment outcomes among people living with HIV diagnosed by Xpert MTB/RIF versus smear in Botswana. Submitted to PLoS One, 03 Jun 2019, under review.
3. Zimba O, Tamuhla T, Basotli J, Letsibogo G, Mathebula U, **Agizew T**, et al. The effect of sputum quality and volume on the yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear microscopy among people living with HIV in Botswana (Pan Afrn Med J. 2019;33:110. doi:10.11604/pamj.2019.33.110.15319).
4. **Agizew T**, Basotli J, Alexander H, Boyd B, Letsibogo G, Auld A, et al. Higher-than-expected prevalence of non-tuberculous mycobacteria in HIV setting in Botswana: Implications for diagnostic algorithms using Xpert MTB/RIF assay. PLoS One 2017 22;12(12):e0189981.
5. **Agizew** T, Boyd B, Auld A, Payton L, Pals S, Lekone P, et al. Treatment-outcomes, diagnostic and therapeutic impact: Xpert versus smear systematic review and meta-analysis. Intr Jr Tuberc Lung Dis 23 (1):82-92. Jan 2019.

Abbreviations

ART	Antiretroviral therapy
BNTP	Botswana National Tuberculosis Program
CDC	United States Centers for Disease Control and Prevention
CRF	Case Report Form
MoHW	Ministry of Health and Wellness
MTB	Mycobacterium tuberculosis
MTBC	Mycobacterium tuberculosis complex
NTM	Non-tuberculosis mycobacteria
NTRL	National Tuberculosis Reference Laboratory
PEPFAR	United States President's Emergency Plan for AIDS Relief
PLHIV	Persons living with HIV/AIDS
POC	Point-of-care
SOP	Standard operating procedure
TB	Tuberculosis
WHO	World Health Organization
XPRES	Xpert Package Rollout Evaluation Study

Definitions of terms*

Cured	A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion
Treatment completed	A TB patient who completed treatment without evidence of failure BUT with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable
Treatment failed	A TB patient whose sputum smear or culture is positive at month 5 or later during treatment
Died	A TB patient who dies for any reason before starting or during the course of treatment
Loss- to-follow-up	A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more
Not evaluated	A TB patient for whom no treatment outcome assigned. This includes cases "transferred out" to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit

** Source: World Health Organization. Definitions and reporting framework for tuberculosis – 2013 revision (updated December 2014), 2013 (WHO/HTM/TB/2013.2; Accessed on 04 September 2016. Available at: http://www.who.int/tb/publications/definitions/en/*

Role of student on the doctoral work:

Dr. Agizew, the PhD candidate is one of the principal investigators (PI) for the Xpert MTB/RIF Package Roll out Evaluation Study (XPRES) trial (registered at clinical trial website: NCT02538952) who conceptualized the study and drafted the first XPRES protocol where the doctoral study was embedded in. Dr. Agizew as senior supervisor was a team lead for XPRES since study inception (August 2011) and was responsible for day-to-day trial management including supervision of over 60 staff members, including doctors, nurses, laboratory manager, laboratory technician, recruitment and retention officers. Dr. Agizew was also responsible for technical aspects of the study that includes designing of the protocol and data collection forms, Institution Review Board reviews, convening co-investigators meetings, training of study staff, abstract and manuscript submission to conferences or publications. Dr. Agizew conducted all aspects (from conceptualizing the idea, writing the protocol, supervised fieldwork, data collection and analysis of data, manuscript and publication) of this PhD study. XPRES trial laboratory team assisted Dr. Agizew with extracting Xpert MTB/RIF result from GeneXpert instrument from the 13 instrument placement sites, testing extra laboratory testing for culture and non-tuberculous mycobacteria speciation. Dr. Agizew authored (first author) of the doctoral study and written all manuscripts. The PhD supervisors and other co-authors included their input in the process for respective manuscripts they contributed to. The only exception was 'the Effect of sputum quality and volume on the yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear' manuscript where Dr. Agizew was a senior author and mentored one of the laboratory technicians to perform the analysis and writing the manuscript.

Executive summary

Background: Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent. Globally, ten percent of TB patients co-infected with HIV and 72% occurred in Africa. Botswana has the second-highest HIV infection rate in the world, with one in five adults infected. The estimated annual incidence of TB was at 385 per 100, 000 population and with a TB/HIV co-infection rate of 60% in 2015 [5]. In Botswana, as in the rest of sub-Saharan African countries, undiagnosed TB or TB diagnosed late in the course of a disease is the leading cause of death among PLHIV regardless of ART status.

The 'End TB strategy' proposes reaching 95% reduction in the number of TB deaths and 90% reduction in TB incidence rate by 2035 compared to 2015. To achieve such a goal people with active TB disease have to be diagnosed and the new molecular test, Xpert MTB/RIF, is a key. The World Health Organization (WHO) endorsed the use of Xpert MTB/RIF as a molecular test for the detection of TB cases in 2010 to support intensified case finding activities. Countries are shifting from smear microscopy (smear)-based to Xpert MTB/RIF-based tuberculosis (TB) diagnostic algorithms. Following the WHO recommendation, the Botswana Ministry of Health and Wellness (MoHW) in 2011 implemented Xpert MTB/RIF at peripheral clinics [referred as point-of-care, (POC)] and centralized laboratories that provided a unique opportunity for to this doctoral study.

The Xpert MTB/RIF Package Roll out Evaluation Study (XPRES), evaluating the accuracy, impact and operational challenges of the GeneXpert use for TB case finding among HIV infected persons, formed the basis for this PhD. Three sub-studies were nested in the XPRES study, evaluating Xpert MTB/RIF implementation in Botswana.

The aim of this PhD was to evaluate the performance of Xpert MTB/RIF in the diagnosis of TB when implemented routinely at a POC and centralized laboratory. The five objectives achieved the above aim through:
(1) To compare the performance Xpert MTB/RIF when implemented under routine conditions at peripheral clinics versus centralized laboratory (Study #1).
(2) To determine the impact of Xpert MTB/RIF on patient-level outcomes (Study #2).
(3) To determine diagnostic and therapeutic factors affecting patient-level outcomes among individuals diagnosed with Xpert MTB/RIF (Study #2).

(4) To determine the effect of appearance (quality) and volume (quantity) of sputum on Xpert MTB/RIF test result (Study #3).
(5) To determine the prevalence of Mycobacteria species from sputum samples collected from patients enrolled in the Xpert MTB/RIF Package Rollout Evaluation Study (Study #4 and 4a). Additionally, we conduct systematic review and meta-analysis on the impact of diagnostic and therapeutic on treatment outcome, comparing Xpert MTB/RIF and smear (Study #5) which is related with objective 2.

Methods:
This PhD achieved through five objectives and used retrospective approaches as part of the parent study, the XPRES Trial.

Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic trial in Botswana: From August 2012 through November 2014. GeneXpert instruments (GeneXpert) deployed in nine centralized laboratories and four point-of-care (POC) peripheral clinics in a phased approach. Clinicians and laboratorians were trained on the four-symptom TB screening algorithm and Xpert MTB/RIF testing. We documented our experience with staff training and GeneXpert performance across the sites. Test results were extracted from GeneXpert software; unsuccessful tests were analyzed in relation to testing sites and trends over time.

Tuberculosis treatment outcomes among people living with HIV diagnosed by Xpert MTB/RIF versus smear in Botswana: From August 2012 - November 2014, PLHIV initiating antiretroviral therapy at 22 HIV care and treatment centers in Botswana were enrolled and underwent systematic screening for TB. GeneXpert instruments were deployed following a stepped-wedge design in 13 sites from November 2012 - June 2013 to serve the centers. National smear and Xpert MTB/RIF intensified case finding algorithms were followed: initially symptomatic patients were evaluated by testing sputum samples using a smear (smear arm) and after GeneXpert instruments were installed, by testing with an Xpert (Xpert arm). TB treatment outcomes identified as unfavorable (death, failure or loss-to-follow-up) and favorable (cure, treatment completion) were compared between patients with drug-sensitive TB diagnosed by smear and Xpert, using cox proportional hazard ratio adjusting for intra-site correlation.
The Effect of sputum quality and volume on the yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear: From August 2012 to November 2014, all people living with HIV

were recruited at 22 clinics. For patients screened positive using the four TB symptoms, their sputa were tested by Xpert MTB/RIF and smear. Laboratorians assessed and recorded sputum appearance and volume. The yield of bacteriologically-positive sputum was evaluated using Xpert MTB/RIF and smear, likelihood-ratios were calculated.

Higher-than-expected prevalence of non-tuberculous mycobacteria in HIV setting in Botswana: Implications for diagnostic algorithms using Xpert MTB/RIF assay: All PLHIV patients presenting for HIV care and treatment services at 22 clinical sites in Botswana were offered screening for TB and recruited. Patients who had ≥1 TB symptom were asked to submit sputa for Xpert MTB/RIF and culture. Culture growth was identified as Non-tuberculous mycobacteria (NTM) and Mycobacterium tuberculosis complex (MTBC) using the SD-Bioline TB Ag MPT64 Kit and Ziehl Neelsen microscopy. NTM and MTBC isolates underwent species identification by the Hain GenoType CM and AS line probe assays.

Treatment-outcomes, diagnostic and therapeutic impact: Xpert MTB/RIF versus smear systematic review and meta-analysis: Citations (2000-2016) reporting treatment-outcomes of patients diagnosed by Xpert MTB/RIF compared to smear were selected from PubMed, Scopus and conference abstracts. We conducted a systematic review and meta-analysis. Favorable (cured, completed) and unfavorable (failure, death, loss-to-follow-up) outcomes were pooled for meta-analysis; we reviewed numbers of TB cases diagnosed, time to treatment and empiric treatment. The Mantel-Haenszel method with a fixed-effect model was used; I^2 was calculated to measure heterogeneity.

Results: This PhD comprised of five studies (1-5) and the results presented accordingly as described in the methods section:

In evaluating the performance of Xpert MTB/RIF, during 276 instrument-months of operation a total of 3,630 tests were performed, of which 3,102 (85%) were successful with interpretable results. Of all 3,630 Xpert MTB/RIF tests, 528 (15%) were unsuccessful; of these 361 (68%) were classified as “error”, 119 (23%) as “invalid” and 48 (9%) as “no result”. Cumulative incidence of unsuccessful test was higher at POC (17%, 95% confidence interval, CI: 11-25%) than centralized laboratory-based GeneXpert instruments (14%, 95% CI: 11-17%; *p=0.14*), though the difference was not statistically significant. The total number of recorded error codes was 385 and the most common reasons were related to sample processing (211; 55%).

For the TB treatment outcomes study, a total of 256 [199 per 2985 person-years and 57 per 1582 person-years of follow-up, in Xpert and smear arm, respectively, adjusted incidence rate ratio, 9.07, 95% confidence interval (CI) 4.70-17.48, *P<0.001*] were diagnosed and were treated for drug-sensitive TB. TB treatment outcomes as favorable or unfavorable were available for 203/256 (79.3%); 157 were in Xpert and 46 in smear arm. Unfavorable outcome was 10/46 (21.7%) for smear and was 21/157 (13.4%) for Xpert arm, adjusted hazard ratio, 1.40, 95% CI: 0.75-2.26, *p=0.27*. Between smear and Xpert arm, respectively, the median number of days (Interquartile Range, IQR) from sputum collection to TB treatment were 22 (3-51) and 6 (2-17), *p*=0.005; empiric treatment was 39/57 (68.4%) versus 97/199 (48.7%), aOR, 2.28, 95% CI: 1.24–4.20, *p=0.011*); and microbiologically-confirmed TB was 18/43 (41.9%) versus 102/173 (59.0%), aOR, 2.00, 95% CI: 1.01–3.96, *p=0.048*).

Thirty-six (14%) of 256 patients received TB treatment despite a positive culture only for NTM and 34 of 36 were also negative by Xpert MTB/RIF.

For the sputum quality evaluation, among 6,041 patients enrolled 2,296 were presumptive TB, 1,305 (56.8%) had >1 sputa collected and 644/1,305 (49.3%) had both Xpert MTB/RIF and smear results. Since >1 sputa collected from 644 patients 954 sputa were tested by Xpert MTB/RIF and smear. Bacteriologically-positive sputum was two-fold higher with Xpert MTB/RIF 11.4% versus smear 5.3%, p<0.001. Sputum appearance and quantity were not predictive of bacteriologically-positive results, except the volume of 2ml to < 3ml, tested by Xpert MTB/RIF LR+=1.26 (95% CI, 1.05–1.50).

The NTM study included 1940 patients who submitted ≥1 sputum specimen and 427 (22 %) patients had ≥1 positive-culture result identified phenotypically for mycobacterial growth. Of these, 247 and 180 patients were identified as having NTM and MTBC isolates, respectively. Of the 247 patients identified with isolates containing NTM; 19 were later excluded as not having NTM based on additional genotypic testing. Among the remaining 408 patients, 228 (56%, 95% confidence interval, 46-66%) were NTM. *M. intracellulare* was the most common isolated (47.8%). Other NTMs commonly associated with pulmonary disease included *M. malmoense* (3.9%), *M. avium* (2.2%), *M. abscessus* (0.9%) and *M. kansasii* (0.4%). After excluding NTM isolates that were non-speciated and *M. gordonae* 154 (67.5%) of the NTM isolates were potential pathogens.

For the systematic review, from 13 citations, 43,594 TB patients were included and 4,825 were with known TB treatment-outcome. From the pooled analysis, the unfavorable outcome among those diagnosed using Xpert MTB/RIF compared with smear was 20.2%, 541/2,675 versus 21.9%, 470/2,150, risk-ratio 0.92, 95% Confidence Interval, 0.82-1.02. Statistical heterogeneity was low (I^2=0.0%, *p=0.91*). Compared to smear, Xpert MTB/RIF was reported to be superior in increasing the number of TB patients diagnosed (2/9 citations), increasing bacteriologically-confirmed TB (7/9 citations), reducing empiric treatment (3/5 citations), reducing time to diagnosis (2/3 citations), and reducing time to treatment initiation (1/5 citations).

Conclusions: From this PhD we demonstrated that Xpert MTB/RIF implementation was feasible in Botswana. With the introduction of Xpert MTB/RIF in the Botswana TB program setting, we presented operational challenges and improvements achieved with a more sensitive molecular test in the diagnosis of TB patients. However, such improvement in detection of TB was not extended to patient-level treatment outcomes. Several factors, including issues around health system, human capacity, laboratory capacity, inadequate TB diagnostic algorithms and quality of sputum specimen were uncovered.

Xpert MTB/RIF introduction was successful in the Botswana setting. There were more unsuccessful tests at the POC compared to the centralized laboratory, though the difference was not statistically significant. This suggests that Xpert MTB/RIF performance was unlikely affected when operated by nurses, environmental factors, power supply at the POC nor the centralized laboratory. However, unsuccessful test incidence (15%) in our settings was higher than previously reported and was mostly related to improper sample processing. Ensuring adequate training among Xpert MTB/RIF testing staff is essential to minimize errors.

Xpert MTB/RIF implementation, as evidenced both in the treatment outcome study and the systematic review/meta-analysis, though not statistically significant, showed reduced unfavorable outcome compared to conventional smear.

Xpert MTB/RIF test yield in bacteriologically-confirmed TB was superior to smear. Sputum quality and quantity, however, were not consistently predictive of bacteriologically-positive results by Xpert MTB/RIF or smear.

In our high HIV prevalent setting, over-half (56%) of the positive sputum cultures from PLHIV, with symptoms suggestive of TB, were NTM. In our study, though we were not able to distinguish between NTM disease and colonization, the study suggests culture and species identification for PLHIV presenting with TB symptoms remains important to facilitate NTM diagnosis and hasten time to appropriate treatment. Since patients with NTM disease present with symptoms similar to those of TB, where the testing capacity for culture and NTM speciation is limited, misdiagnosis, delivery of sub-optimal treatment and outcome may occur.

Further research is warranted to identify gaps in the existing health system, and NTM disease as a potential factor affecting treatment outcome.

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My sincere thanks also go to bigger XPRES research team who provided me an opportunity to join their team as XPRES research team lead and supervisor of the research team, and also gave me access to the laboratory and research facilities where my PhD was conducted. Without their precious support, it would not have been possible to conduct this research.

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CHAPTER 1: INTRODUCTION

1.1. Background

The Xpert MTB/RIF assay recommended as an initial diagnostic test among people living with human immunodeficiency virus (PLHIV)-associated presumptive tuberculosis (TB) or multi-drug resistant TB (MDR-TB) [1]. After endorsement by the World Health Organization (WHO) in 2010, over 145 countries have implemented the assay as of December 2016 [2]. Botswana is one of the countries that implemented Xpert MTB/RIF assay both at peripheral clinics (referred hereafter as point-of-care, POC) and centralized laboratory. Embedded in the Xpert MTB/RIF implementation was the Xpert Package Rollout Evaluation Study in which this PhD was nested.

1.1.1. Epidemiology of TB and TB/HIV co-infection

Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent. Millions of people continue to fall sick with TB each year. An estimated 10 million people globally developed TB disease in 2017, 90% were adults aged ≥15 years, 9% were PLHIV and 72% occurred in Africa [3]. In the same year, there were an estimated 1.3 million deaths among HIV-negative people and an additional 300 000 deaths from TB among HIV-positive people [3]. Most of the gaps in detection and treatment were in the WHO African Region, where the burden of HIV-associated TB is highest [3].

Botswana has the second-highest HIV infection rate in the world, with one in five adults infected [4]. In 2015, the estimated annual incidence of TB was at 385 per 100, 000 population with a TB/HIV co-infection rate of 60% [5]. In Botswana, as in the rest of sub-Saharan African countries, undiagnosed TB or TB diagnosed late in the course of a disease is the leading cause of death among PLHIV regardless of ART status. [6-9]. Failure to diagnose TB might be due to: (i) the difficulty of appropriately screening HIV-infected patients for TB [10]; (ii) difficulty in diagnosing TB with traditional diagnostic methods such smear microscopy (smear) [11-13]; and (iii) sub-clinical TB that is missed by standard screening algorithms [11].

1.1.2. The end TB strategy

Xpert MTB/RIF within the context of end TB strategy:

The WHO laid out an ambitious goal of the end TB strategy: (1) compared with 2015 to reach 95% reduction in the number of TB deaths, 90% in TB incidence rate and Zero TB-affected families facing catastrophic costs due to TB by 2035. To realise this vision integrated, patient-centred care and prevention strategies with early diagnosis of TB and systematic screening of contacts and high-risk groups recommended as one of the pillars [14].

To intensify research and innovation the WHO advocate for discovery, development and rapid uptake of new tools such as Xpert MTB/RIF, interventions and strategies. To that effect, to optimize implementation and impact, and promote innovations research remains as essential components of the end TB strategy [14]. XPRES, in which the doctoral study is nested is an implementation trial in an attempt to evaluate impact of Xpert MTB/RIF (potential patient level treatment outcomes benefits) and operational challenge in program settings.

With the current trend, the decline in global TB incidence rates remains at 1.5% per year. With the optimization of current tools combined with progress towards universal health coverage and social protection, the 5-10% yearly TB incidence reduction might be achieved by 2035 [14]. However, the incidence rate remains above 25 per 100, 000, making it challenging to reach the TB elimination goals. That is the reason why the introduction of new tools such as Xpert MTB/RIF test, particularly at a POC, is highly needed to reach the 2035 goal, where TB incidence rate reduced to <10 per 100,000 population. With such combined effort, including new TB drugs and treatment regimen and TB preventive therapy, the 17% TB incidence reduction is estimated that provides hope for TB elimination by 2035 [14].

1.1.3. Diagnosis of TB using Xpert MTB/RIF

Without an early and accurate diagnosis, TB is impossible to control. Historically, TB control programs have relied on sputum smear as the main diagnostic tool for active TB [15]. Even in 2016, smear remained the most widely used test in low and middle-income countries (LMIC). Unfortunately, a smear is neither sensitive nor able to detect drug resistance. More recently, molecular diagnostics, with higher sensitivity, specificity and that can be performed within hours allowing for faster turn-around time, are starting to be implemented even in LMICs.

WHO has endorsed three rapid nucleic acid amplification tests (NAATs) since 2008: line probe assays (LPA) for detection of TB and resistance to first- and second-line TB drugs, Xpert MTB/RIF, an automated, cartridge-based assay, and a manual TB-LAMP assay, based on isothermal amplification process for detection of *Mycobacterium TB* complex (MTBC). Implementation research is essential: (a) to validate more accessible and affordable POC molecular diagnostics at lower tiers of health care with efficient systems for sample referral, and (b) to establish patient-level-benefits of molecular diagnostics [16].

1.1.3.1. Implementation of Xpert MTB/RIF

The Xpert MTB/RIF is a real-time fully automated molecular test, developed on the GeneXpert platform (Cepheid, Sunnyvale, CA, USA), that can detect both MTBC and rifampicin (RIF) resistance within two hours [17, 18]. The GeneXpert instrument (GeneXpert) operates with modules that are the heart of the analytic system, using patented cartridge-based technology [19]. The module currently on the market uses a new software and cartridge version, in combination referred to as G4, that has a potential for reducing non-determinant results and error rates than the earlier version, G3[20].

In 2011, the Botswana Ministry of Health and Wellness (MoHW) adopted WHO guidelines and incorporated Xpert MTB/RIF into the national TB diagnostic algorithm [13]. With Xpert MTB/RIF Package Roll out Evaluation Study (XPRES) by the US Centers for Disease Control and Preventions (CDC) initial Xpert MTB/RIF implementation was introduced [21].

The WHO advocates for the swift and large-scale implementation of Xpert MTB/RIF because of its improved diagnostic performance over smear [22]. However, the readiness of intended users and the health system at the implementation level POC or laboratory sites is unclear. A 2014 Cochrane review by Steingart *et al* acknowledged that not all district or sub-district-level laboratories might be able to satisfy the operational requirements recommended for Xpert MTB/RIF, namely an uninterrupted and stable electrical power supply, temperature control, and yearly calibration of the instrument modules [23]. From early program implementation in nine high TB burden countries, a wide range of challenges such as Xpert MTB/RIF performance, infrastructure, training, recording and reporting were reported [24]. Furthermore, Durovni *et al* reported that because of operational challenges substantial

controversies remain about where GeneXpert should be placed (peripheral clinics versus centralized laboratories) [25].

Some elements around its implementation, however, may have limited the potential impact of Xpert MTB/RIF. Depending on the gaps in the implementation, the impact of Xpert MTB/RIF may vary from profound to almost imperceptible [26]. Hence, implementing countries should give due consideration on evaluating the potential implementations hurdles.

As a new intervention, the implementation of Xpert MTB/RIF is unlikely to happen without challenges. Challenges may be faced at various points including; initial introduction, normalizing and sustaining Xpert MTB/RIF use until it is embedded in a setting such that it becomes "normal" practice [27]. Rachow *et al* described that the expected impact of Xpert MTB/RIF will depend on the reproducibility of the results in routine health settings, the manner and extent of their introduction, the strength of the laboratories and the degree to which access to appropriate therapy follows access to diagnosis [28]. The diagnostic and therapeutic factors that have a potential to affect the intended Xpert MTB/RIF impact are shown below (Fig. 1).

1.2. Justification of the PhD study

As more countries, particularly low- and middle-income countries, expand the initial implementation of Xpert MTB/RIF, the evaluation of Xpert MTB/RIF performance is essential. There are limited data that compares Xpert MTB/RIF performance when placed at a peripheral clinic operated by a nurse versus a laboratory sites operated by a laboratorian. From the few available reports, considerable controversies remain on the placement of GeneXpert posed by operational challenges, and the arguments remain the same in Botswana, as well. The findings of this study will be useful in guiding policy in Botswana and other low- and middle-income countries in terms of GeneXpert placement and operators, nurses versus laboratorian lead diagnosis.

In Botswana, since the initial implementation of Xpert MTB/RIF for TB diagnosis there is little data on Xpert MTB/RIF performance in routine care settings, including failure rate, detailed error rate stratified by GeneXpert placement, performance over time and effect of delayed instrument calibration on test performance.

Additionally, comparing smear and Xpert MTB/RIF diagnostic and therapeutic impact this thesis elucidates and help to understand patient-level clinical outcomes within the local context, especially at decentralised peripheral clinic settings.

The current Xpert MTB/RIF algorithm does not address Xpert MTB/RIF negative and NTM culture-positive patients. Isolation of NTM may have implications Xpert MTB/RIF diagnostic algorithms and management of Xpert MTB/RIF-negative clients that have symptoms suggestive of TB. Furthermore, data on the specificity of Xpert MTB/RIF among NTM positive cultures in programmatic HIV setting in Botswana and other sub-Saharan Africa countries is lacking.

1.3. Research questions

This PhD addresses the following key questions:

- How do new diagnostic tests such as Xpert MTB/RIF perform when implemented in routine peripheral sites in high TB and HIV prevalence settings and what other operational and performance considerations should be made?

- Compared to smear, how does Xpert MTB/RIF assay affect diagnostic performance and impact therapeutic outcomes?

The following aims and objectives addressed the research questions:

1.4. Thesis Aim

To evaluate the performance of Xpert MTB/RIF in the diagnosis of TB when implemented at peripheral clinics compared to centralized laboratories in routine programmatic HIV settings.

1.5. Thesis Objectives

1. To compare the performance Xpert MTB/RIF when implemented under routine conditions at peripheral clinics versus centralized laboratory.

2. To determine the impact of Xpert MTB/RIF on patient-level outcomes.

3. To determine diagnostic and therapeutic factors affecting (impacting) patient-level outcomes among individuals diagnosed with Xpert MTB/RIF.

4. To determine the effect of appearance (quality) and volume (quantity) of sputum on Xpert MTB/RIF test result.

5. To determine the prevalence of Mycobacteria species from sputum samples collected from patients enrolled in the Xpert MTB/RIF Package Rollout Evaluation Study.

1.6. Themes organization of thesis

Themes	**Studies**					
	Cover story	**I** **Performance of Xpert MTB/RIF at point-of-care versus laboratory**	**II** **TB treatment outcome, Xpert MTB/RIF versus smear**	**III** **Effect of sputum quality and volume on confirmed TB by Xpert MTB/RIF and smear**	**IV** **Prevalence of NTM and implications for diagnostic algorithms using Xpert MTB/RIF assay**	**V** **A systematic review and meta-analysis Treatment-outcomes, diagnostic and therapeutic impact: Xpert MTB/RIF versus smear**
Evaluating the operational performance of Xpert MTB/RIF when implemented at peripheral clinics	Lack of pragmatic, field level information: Xpert MTB/RIF performance and operational challenges within Botswana context; then based on lesson learned contribute to National scale up	Identified operational challenges, performance level and result used for national scale up	Treatment outcome not significantly improved by molecular test, gap in a health systems need further evaluation			
Determining the impact of Xpert MTB/RIF on patient-level treatment outcome	Critical examination of impact of molecular assay on patient level outcome compared to conventional smear	Treatment outcome not significantly affected by placement of Xpert MTB/RIF, at point-of-care or laboratory.	With Xpert MTB/RIF no significant treatment outcome improvement, despite reducing time to treatment and empiric treatment	Sputum quality and quantity concerning, especially with high proportion of sub-optimal (salivary) sample	Concerning that NTM patients with Xpert MTB/RIF negative test received anti-TB treatment. Xpert MTB/RIF based algorithm need to address NTM patients	Health system matters, shifting smear to Xpert MTB/RIF alone is not enough

Themes	**Studies**					
	Cover story	**I** **Performance of Xpert MTB/RIF at point-of-care versus laboratory**	**II** **TB treatment outcome, Xpert MTB/RIF versus smear**	**III** **Effect of sputum quality and volume on confirmed TB by Xpert MTB/RIF and smear**	**IV** **Prevalence of NTM and implications for diagnostic algorithms using Xpert MTB/RIF assay**	**V** **A systematic review and meta-analysis Treatment-outcomes, diagnostic and therapeutic impact: Xpert versus smear**
Mycobacterium prevalence and Xpert MTB/RIF specificity	Establishing NTM and MTBC prevalence among PLHIV		Discovered that NTM patients received anti-TB treatment. Concern for lack of appropriate NTM specific treatment	Sputum quality concerning	NTM prevalence established, specificity of Xpert MTB/RIF reported. National TB diagnostic algorithm change recommended	
Implications of sputum volume and quality on yield to MTB using Xpert MTB/RIF	Does quality of sputum specimen matter?		Yield to MTB negatively affected by quality of sputum and thus implication on TB diagnosis and treatment outcome	Sputum quality and quantity, were not predictive of bacteriologically-positive results by Xpert MTB/RIF or smear		
Systematic review and meta-analysis	How diagnostic and therapeutic factors affect impact of Xpert MTB/RIF		Overall no impact on patient-level TB treatment outcome observed			No discernible impact on treatment-outcome compared to conventional smear despite reduced time to diagnosis, time to treatment or reduced level of empiric treatment.

Note: NTM – non-tuberculous mycobacteria; MTB - *Mycobacterium tuberculosis* complex;

1.7. Conceptual Framework

As principal investigator, I was running the XPRES trial where the primary intent was to conduct implementation research while supporting Botswana on initial introduction of Xpert MTB/RIF. Conceptualizing the PhD work began while evaluating Xpert MTB/RIF based TB diagnostic algorithm as part of the XPRES trial. The observed potential factors affecting the performance of Xpert MTB/RIF led to the development of concept paper underscoring the need to evaluate not only the test performance but also the GeneXpert instrument, a human capacity that operates the instrument and the health systems. Following Xpert MTB/RIF implementation, improved patient- outcomes were expected and this PhD conceptual framework included the components involved in the process from Xpert MTB/RIF implementation through treatment outcome improvement as indicated in Fig.1.

Improving TB diagnosis may lead to improved patient outcomes. However, the programmatic implementation of new diagnostics such as Xpert MTB/RIF will require consideration of three key components: (1) *instrument performance, human resources, and health system factors.* Botswana, a high TB and HIV burden settings is looking to improve outcomes especially in those co-infected with HIV through the provision of improved diagnostics. Fig.1. shows the conceptual framework for this PhD, representing key considerations when implementing a new diagnostic test, such as Xpert MTB/RIF in order to maximise patient outcomes.

The success of the implementation can be affected by:

(1) Performance of the GeneXpert Instrument. Environmental factors such as temperature, uninterrupted electric power supply, and yearly instrument calibration affect instrument performance.

(2) Availability of trained human capacity to identify patients that need to have sputum collected for TB diagnosis, refer and initiate appropriate management of patients after a diagnosis is made, operate a GeneXpert instrument and interpret the test result.

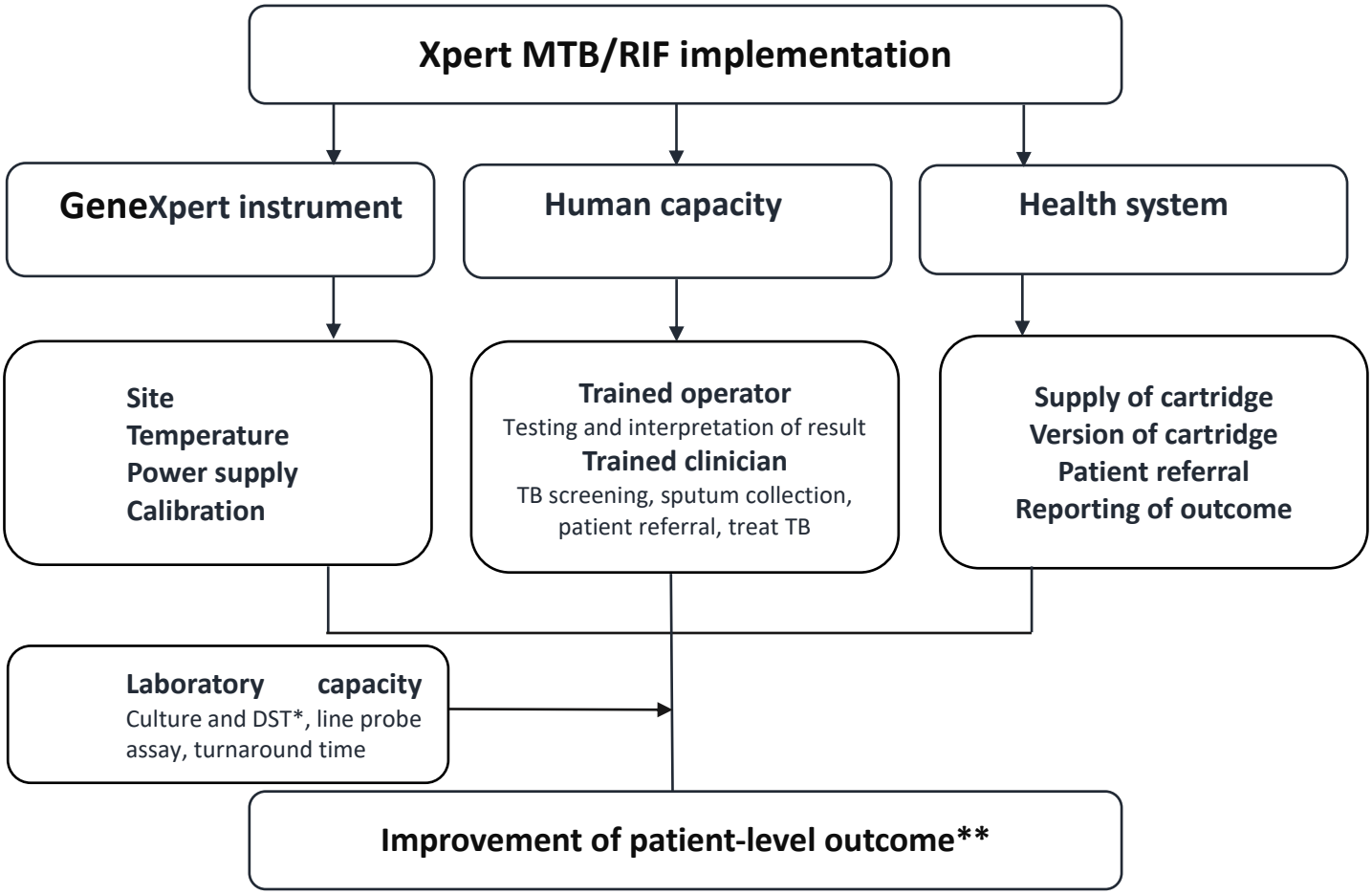
(3) A health system that caters a range of systems from an uninterrupted supply of Xpert MTB/RIF cartridge with latest available cartridge version, a patient referral from diagnostic

to treatment centers, especially when rifampicin-resistant TB identified, and finally documenting and reporting of patient-level TB treatment outcome. Additionally, as further diagnostic tests such as culture and drug susceptibility testing (DST) using either culture or line probe assay, maybe needed, they were included in the framework.

As described above, each of the major components in the conceptual framework directly or indirectly affect patient-level treatment outcomes. If improvement to patient-level-treatment outcomes were to be achieved, Xpert MTB/RIF testing need to be conducted by trained personnel who passed a proficiency test and perform with expected minimum error rates, screening for TB be conducted per the national guidelines and quality sputum samples collected, transported and tested timely. Once test results are available, they need to be reported to a treating clinician for appropriate management. Patients presenting with NTM disease and symptoms suggestive of TB may require further species identification and be managed appropriately.

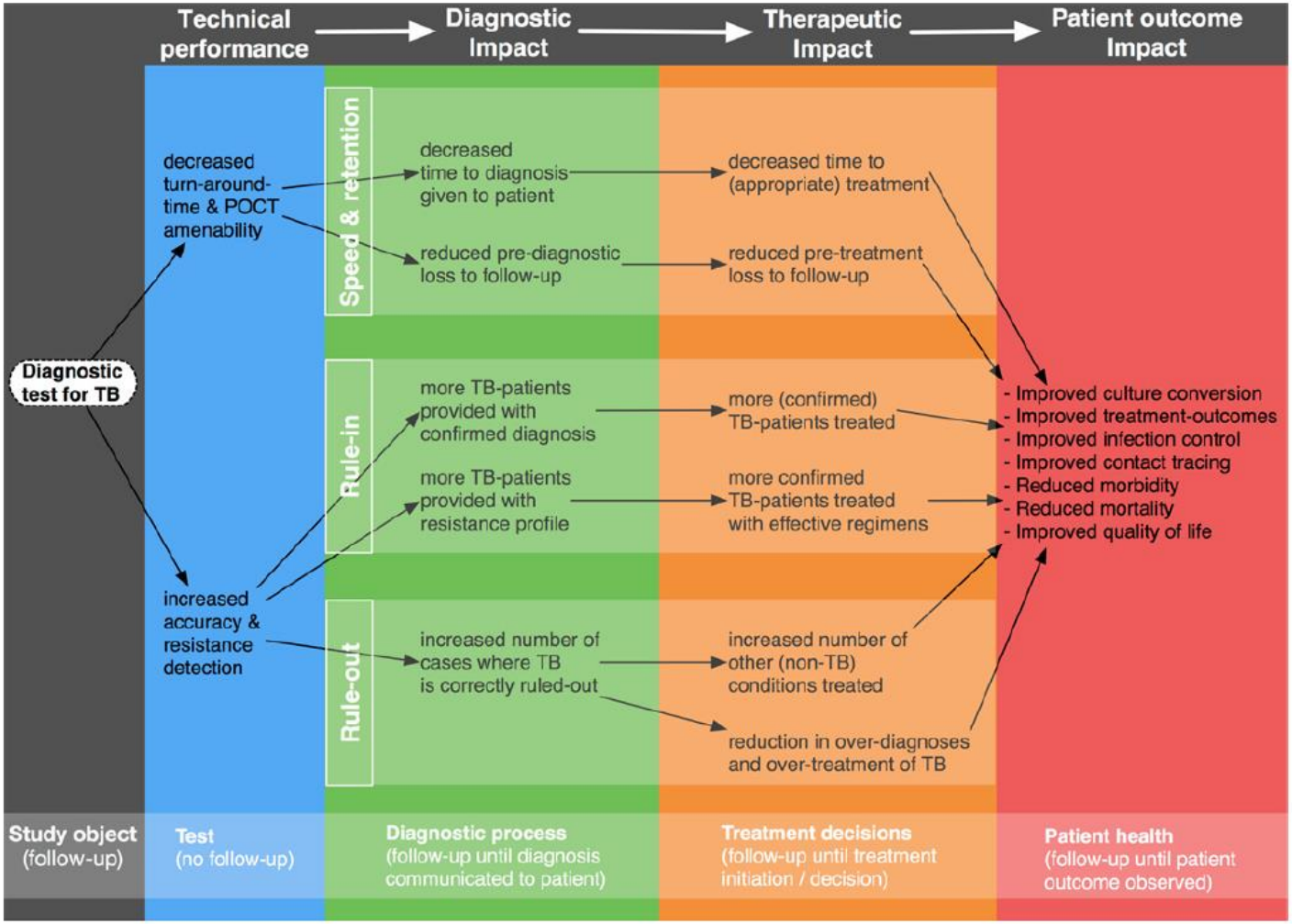
Each of these components was evaluated and addressed in the PhD. GeneXpert instrument performance, the effect of human capacity and the health system were covered in paper1; Improvement to patient-level treatment outcome with an effect of a trained clinician in diagnosing and treating patients was covered under TB treatment outcome manuscript (paper 2). The systematic review and meta-analysis publication (paper 5) covered overall treatment outcome in relation to the diagnostic and therapeutic impact of Xpert MTB/RIF; higher than expected NTM publication (paper 4) covered laboratory capacity related components. The conceptual framework developed with this PhD in 2015 was consistent with that of Schumacher *et al* published in 2016 (Fig. 2) [29]. The PhD conceptual framework additionally included few more components (e.g. human capacity – training, health system – supply of cartridge and version, local lab capacity) than Schumacher *et al*. (Schumacher *et al* published their framework after approval of the conceptual framework for this PhD).

Fig. 1. Conceptual framework for implementation of Xpert MTB/RIF that may lead to improved patient outcomes



* DST = Drug susceptibility and testing
** Reduce time to diagnosis, time to treatment initiation, reduce empiric treatment, reduce loss to follow up and increase bacteriologically-confirmed TB. As a result reduce unfavourable outcome including mortality.

Fig. 2. Outline the pathways through which improved TB diagnostics may lead to improved patient outcome.



Source: Schumacher et al PLoS One, 2016, doi:10.1371.pone.0151073.g002. [29]

1.8. Structure of the integrated narrative

Chapter 1 has introduced the background for implementing Xpert MTB/RIF assay following the WHO endorsement in 2010. A brief background of Botswana TB and HIV situation was included and how Xpert MTB/RIF assay introduced with XPRES study. The rationale and the conceptual framework that was used for the PhD study has been described.

Chapter 2 reviews the relevant literature on Xpert MTB/RIF as a new TB diagnostic molecular technology and the potential to positively affect patient-level treatment outcome.
Chapter 3 describes the methodologies used in this PhD.
Chapter 4 presents the results of the PhD under the following headings:

- Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic trial in Botswana
- Tuberculosis treatment outcomes among people living with HIV diagnosed by Xpert MTB/RIF versus smear in Botswana
- The Effect of sputum quality and volume on the yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear
- Higher-than-expected prevalence of non-tuberculous mycobacteria in HIV setting in Botswana: Implications for diagnostic algorithms using Xpert MTB/RIF assay
- Treatment outcomes among people living with HIV with mycobacterium species, comparing non-tuberculosis mycobacteria versus Mycobacterium tuberculosis in Botswana
- Treatment-outcomes, diagnostic and therapeutic impact: Xpert MTB/RIF versus smear systematic review and meta-analysis

Chapter 5 includes the discussions, conclusions of the PhD findings and recommendations

CHAPTER 2: LITERATURE REVIEW

The Global and Botswana TB and TB/HIV burden, TB diagnostic gap with the less sensitive conventional smear and the need for advanced affordable and accessible TB diagnostics was covered under introduction. In addition, the importance of early and accurate diagnosis of TB was emphasized for the end TB strategy to materialize.

This literature review focused on what this PhD addresses and with that described the in-depth current knowledge.

Xpert MTB/RIF is a new TB diagnostic technology and several clinical studies demonstrated its usefulness for intensified case finding [30, 31]. Emerging evidence about GeneXpert instrument performance in programmatic settings has shown variability of performance in failure (error, invalid or no result) rates and accuracy [32-34].

First, following the WHO endorsement of Xpert MTB/RIF in 2010, many countries increased the laboratory capacity by implementing Xpert MTB/RIF, to screen and diagnose TB and are shifting from smear based diagnostic algorithms [35]. Because of the potential hurdles, evaluating the introduction of Xpert, its performance in real-world (programmatic or routine) HIV settings, particularly when used in a decentralized health system is recommended [28].

Recent publications, mostly involving multiple countries with high TB burden in Africa, Asia and Europe, have demonstrated overall Xpert MTB/RIF failure rate of 5-10% [24, 32, 36, 37]. Two of these studies reported Xpert MTB/RIF results from laboratory settings [31, 37]. The other two included peripheral clinics in their report, one programmatic [24] and the other in a controlled trial setting [36]. Though Creswell *et al* included peripheral clinics in their study, skilled laboratory technician conducted Xpert MTB/RIF testing, not the recommended non-laboratory operator for POC, and analysis was not stratified by sites [24]. GeneXpert placement and its performance at peripheral clinics versus centralized laboratories is a controversial subject [25]. While countries continue expanding initial implementation, it is important to make an informed decision to reap the full benefits of GeneXpert investment. Evaluating Xpert MTB/RIF performance in programmatic settings is a priority since there is insufficient evidence to justify its implementation.

Apart from the integrity of the GeneXpert instrument itself, a level of GeneXpert instrument operators training, maintaining trained operators (retaining site capacity) over time may also affect Xpert MTB/RIF performance. Creswell *et al* and Adrizzoni *et al* emphasized the need for retraining of GeneXpert operators due to staff turnover [24, 37].

Although it is a possibility to have delayed calibration and test failure in programmatic settings, data on effect of delayed calibration on Xpert MTB/RIF performance, trend on failure rate over time, failure rate stratified by error, invalid and no-result is not available.

Second, based on its accuracy, shorter turnaround time and reduced initial lost to follow-up, Xpert MTB/RIF is expected to result in earlier diagnosis and early anti-TB treatment (ATT) initiation to positively impact patient level clinical outcomes (improve outcome – cured and completion rates and decrease - failure, death and default rates) [25, 38, 39]. In two studies done in South African and Brazil patient level clinical outcomes were found to be similar among patients diagnosed with Xpert MTB/RIF versus smear [38, 40]. More research on patient level clinical outcomes of drug sensitive TB diagnosed by Xpert MTB/RIF versus smear in programmatic settings is needed. Factors associated with favourable (cured and completed) or unfavourable (failure, death and default rates) outcome, such as time to diagnosis and TB treatment initiation, lost to follow-up rate, CD4 count and empirical treatment (*initiating treatment* in absence of or prior to any positive diagnostic test) [41] need to be investigated further. Schumacher *et al* framework displayed in Fig. 2 showed the cascade of diagnostic and therapeutic impact resulting from Xpert MTB/RIF implementation [29].

There was no previous systematic review or meta-analysis conducted comparing drug-sensitive TB treatment outcome between Xpert MTB/RIF and smear.

Third, one of the major reasons implicated for failure to observe difference in clinical impact among presumptive TB patients was empiric treatment for MTBC [37, 41, 42]. Churchyard *et al* reported potential of Xpert MTB/RIF minimizing proportion of patients receiving empiric treatment [43]. Few studies, however, reported on Xpert MTB/RIF negative results in relation to supporting clinical decisions for cessation of empirical ATT or the safe initiation of other treatments [44] such as treatment for non-tuberculous mycobacteria (NTM) that has similar symptoms to TB. Treating Xpert MTB/RIF negative NTM with ATT may negatively affect clinical outcome since NTMs does not or partially respond, depending on species, to ATT. Emerging evidence suggests that NTM rate among PLHIV is increasing in Africa and other parts of the world [45, 46]. Recent reports from some laboratories in sub-Saharan African countries indicates that NTM prevalence among culture positive samples showed NTM prevalence ranges from 4-39% [36, 44, 45, 47-49]. Little data exists on the NTM species

distribution in pulmonary specimens in Africa [50]. Prevalence of NTM among presumptive TB patients or culture positive specimens is unknown in Botswana and given the second highest HIV rate in the world determining a prevalence of NTM infection and disease is a high priority to the national TB control program.

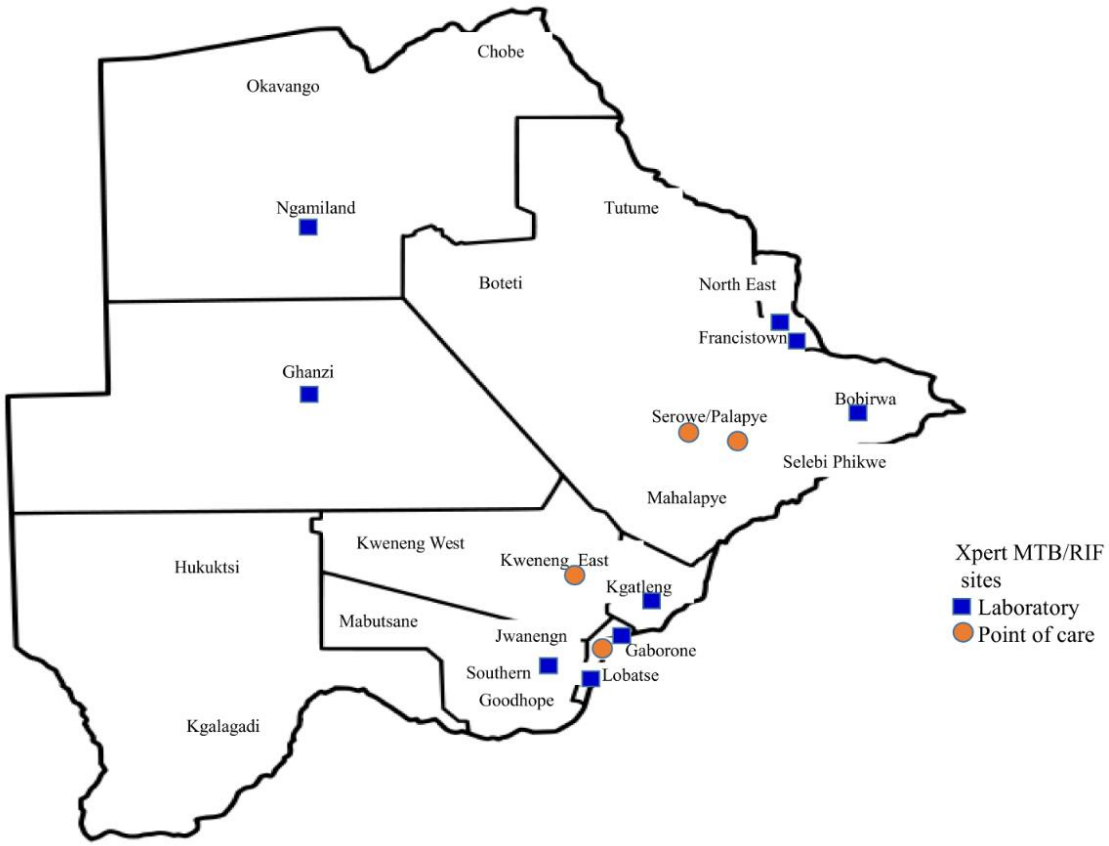
CHAPTER 3 METHODOLOGY

The PhD used retrospective approaches as part of the parent study, the XPRES study. The relation of the PhD to the parent study, study population and data sources are shown in Appendix 2.

3.1. Brief overview of XPRES Study

The XPRES study was a stepped-wedge cluster randomized trial conducted in HIV care and treatment clinics in Botswana to evaluate an Xpert-based TB diagnostic algorithm for new adult HIV clinic enrolees and its impact on mortality. The trial was conducted in 22 HIV care and treatment clinics, in 12 districts (Appendix 3). Thirteen GeneXpert instruments installed over nine months between October 2012 and June 2013 at nine centralized laboratory sites and four POC clinical care sites without on-site laboratory services (Fig. 3). One to two GeneXperts were installed per month during this period, each with four modules for a total of 52 modules (Fig. 3).

Fig. 3. Thirteen GeneXpert instruments sites in Botswana



***Source: Agizew T et al**. Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic, stepped-wedge trial in Botswana. PLoS One 2017 17;12(8):e0183237.*

In Botswana twelve (out of the 28) health districts with high HIV and TB burden were prioritized by the MoHW for initial Xpert MTB/RIF implementation in routine settings. This followed the Botswana National TB guideline revision in 2011 to include Xpert MTB/RIF in the TB diagnostic algorithm. From August 2012 through November 2014, together with patient enrolment and follow-up, Botswana introduced Xpert MTB/RIF in a phased manner and prospectively evaluated the implementation.

Demographic data were collected at enrolment for participants enrolled in the XPRES study. Data on laboratory test results, TB diagnosis, treatment and treatment outcome were collected by study nurses using XPRES case report forms (CRF) at follow up visit. XPRES data clerks entered the data in Clindex XPRES database where data were imported to STATA (STATA Corporation, 2015 statistical software: Release 14. College Station, Texas: StataCorp LP) for analysis [51].

Randomisation procedure:

The selected clusters (n=22) received TB diagnostic services from 13 laboratories. The randomization was done for placement order (as in first, second, etc.) of GeneXpert instrument and not at individual patient-level. In our cluster, four GeneXpert instrument served nine HIV clinics as POC and another 13 HIV clinics were served by nine GeneXpert placed at centralized laboratory.

After obtaining ethical approvals and agreement to participate in the study from MoHW at a central level and health management at the selected facilities, the study statistician randomly selected one of the GeneXpert instrument roll-out permutations. Over the nine months (from October 2012-June 2013), the entire 13 GeneXpert instrument sites were activated and were serving the 22 HIV care and treatment clinics.

Different methodologies used to address the objectives of this PhD from the five studies. We described the detailed data collection and analysis methods for each PhD studies (1 to 5) below.

3.2. Data sources

For this PhD we used data from the following sources:

1. GXX file from GeneXpert, laboratory supervisory report and GeneXpert logistic report used for: Operational performance of GeneXpert and Xpert MTB/RIF testing, and the study was conducted from August 2012 - November 2014.
2. XPRES database (CLINDEX) was used to source data on demographic and clinical, laboratory report (smear and Xpert MTB/RIF results) and TB register report. For the tuberculosis treatment outcomes study, we used these data, and the study was conducted from August 2012 - November 2014.
3. XPRES database was used to source data on sputum quality and volume, and laboratory report including yield of bacteriologically-confirmed TB. For the effect of

sputum quality and volume on yield of yield of bacteriologically-confirmed TB we used these data, and the study was conducted from August 2012 - November 2014.

4. DISA database at the national TB reference laboratory was used to source data on LPA speciation and Xpert MTB/RIF testing for all culture positive results (MTBC, NTM) that we used for: For Higher-than-expected prevalence of non-tuberculous mycobacteria study and Treatment outcomes among people living with HIV with mycobacterium species study we used these data, and the study run from August 2012 - November 2014.
5. PubMed and SCOPUS, for studies published from January 2000 to December 2016, were used to source data on Treatment outcomes, diagnostic and therapeutic impact: Xpert MTB/RIF versus smear systematic review and meta-analysis, the study was conducted from August 2012 to December 2016. We also reviewed abstract books from the Union World Conference on Lung Health from 2012 to 2016 for studies that may have been completed but not published yet.

3.3. Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic trial in Botswana

The methodological approaches described hereunder are per published report (appendix 4) [52].

3.3.1. Setting

HIV care and treatment clinics in 12 districts in Botswana. Thirteen GeneXpert instruments were place in the laboratories as follows: i) nine at centralized laboratory sites in eight districts and ii) four at the POC clinical care sites in four districts without on-site laboratory services (Fig. 3) (Appendix 3) [52].

3.3.2. Study population

The study population includes HIV positive patients presenting for HIV care and treatment at HIV clinics, regardless of age [21].

3.3.3. Sample size

All the Xpert MTB/RIF testing conducted (n=3,630) between August 2012 and November 2014 were included in the analysis. The estimated sample size per protocol was 870 as described below.

Based on Cepheid acceptable level of failure, 5%, and estimated failure rate, 10%, found in XPRES the below sample size would have detected if there was a difference between the two proportions.

Estimated sample sizes for a two-sample proportions test
Pearson's chi-squared test
Ho: p2 = p1 versus Ha: p2 not = p1

Study parameters:
Alpha = 0.05, power = 0.80, delta = 5% (difference)
p1 = 5% and p2 = 10%
Estimated sample sizes: N = 870, N per group = 435

3.3.4. GeneXpert instrument installation

The GeneXpert initially installed by a local GeneXpert vendor who provided calibration and maintenance services during the study, Xpert MTB/RIF cartridge sales, and cartridge delivery services. Air conditioning units installed with each GeneXpert to ensure control of room temperature. Un-interruptible power supply (UPS) systems installed with each GeneXpert to ensure sufficient electrical power to allow completion of in-process sample analysis during power grid outages (up to 30 minutes at sites with back-up generators and up to 2 hours at sites without generators). Xpert MTB/RIF cartridges were procured through the same vendor and the cost covered the cost of the cartridge, central warehousing, and delivery to sites when requested [52].

3.3.5. Training of clinical staff and XPERT MTB/RIF operators

With introduction of Xpert MTB/RIF at implementing districts, training was organised for clinicians and GeneXpert operators. Training for clinicians consisted of a one-day training curriculum and covered standard TB symptom screening, use of the Xpert-based TB diagnostic algorithm [13, 53] and other aspects as previously described [52]. Training for GeneXpert operators consisted of a three-day curriculum and standard operating procedure (SOP) manual as described previously. Briefly, three to four laboratorians at each laboratory and similar number of nurses at POC sites trained as operators. The training covered the theoretical basis of the Xpert MTB/RIF test, operation of the instrument, interpretation of results, troubleshooting, and GeneXpert maintenance (daily, weekly, and monthly). On the third day prior to testing patient specimens from study sites, all trainees had to pass a competency test.

3.3.6. Data collection

As previously describe, to support the implementation, laboratory supervisor or senior study staff member conducted monitoring visits to implementing sites on a quarterly basis. Furthermore, the laboratory supervisors conducted monitoring visits at GeneXpert sites quarterly and during these visits, provided in-service training as needed. Study nurse supervisors conducted such visits to clinical sites monthly [52].

Data were collected by study staff between August 2012 and November 2014 [52]. Xpert MTB/RIF test result files (.gxx file format) were archived monthly by GeneXpert operators, extracted from GeneXpert software, and collected quarterly when laboratory supervisors from the referral laboratory were conducting supervisory visits [52]. GeneXpert software version 4.0 was used to access test results at a central location and results were compiled quarterly and stored in a Microsoft Excel spreadsheet [52]. Tests conducted for quality assurance or training purposes were excluded from the analysis. Unsuccessful test was defined as any result of “error”, “invalid” or “no result”. All remaining tests, which included results as MTBC not detected, MTBC detected plus RIF resistance detected/not detected/intermediate, results were considered successful [52]. The frequencies of successful and unsuccessful tests were analyzed and described, including the type of unsuccessful test

by site, by Xpert MTB/RIF cartridge version (G3 versus G4), and by quarter of operation [52].

3.3.7. Data analysis

Descriptive statistics were computed in Microsoft Excel and in STATA (STATA Corporation, 2015 statistical software: Release 14. College Station, Texas: StataCorp LP) [51]. Robust standard errors were estimated and used in 95% confidence intervals (CIs) for percentages and means. We tested for differences between groups (e.g. POC versus centralized laboratory tests) using the STATA GLLAMM [54] procedure to fit logistic regression models with clinic as a random effect. Interaction variables were included in these models to test for differential effects over time between POC and centralized laboratory tests.

3.4. Tuberculosis treatment outcomes among people living with HIV diagnosed by Xpert MTB/RIF versus smear in Botswana.

3.4.1. Setting

The study was a sub-study of XPRES conducted in the same sites as described in section 3.1.

In summary, we conducted a multi-center, stepped-wedge cluster randomised trial (CRT) called the Xpert Package Rollout Evaluation using a Stepped-wedge design (XPRES) trial. A senior statistician was engaged, and we used appropriate methodology for stepped-wedge as described in the published protocol. To estimate power, data were simulated according to the stepped-wedge design, using the beta-binomial model to induce the intra-cluster correlation coefficient. One thousand datasets were simulated and a mixed model appropriate for the stepped-wedge design fit to the data, as described by Hussey and Hughes [55], that included fixed effects for time and intervention condition (0 for time points before Xpert implementation and 1 afterward), and a random effect for the clinic, to take into account between-clinic variability. This study is a sub-study of the main XPRES study. The details including study populations, sample size, and study procedures were included in the published XPRES protocol [21].

In XPRES, a cluster was defined as an HIV care and treatment clinic. Twenty-two clusters, located at five district hospitals and 17 primary healthcare facilities, were purposively selected to: (1) be representative of HIV treatment clinics in Botswana, and (2) have new ART initiation rates sufficient to meet sample size requirements per protocol. At these 22 clusters, individual patients were eligible for study enrollment if they were new HIV clinic attendees and not prisoners at the time of the first HIV clinic visit from August 2012–November 2014. All eligible patients were enrolled in two consecutive phases: (1) a prospective phase where smear-based TB diagnostic algorithm was used (smear group), and (2) a prospective phase whereby Xpert MTB RIF based TB diagnostic algorithm was used (Xpert group).

Thirteen GeneXpert instruments provided services for the 22 HIV care and treatment clinics, nine at centralized laboratory sites in eight districts and four POC at clinical care sites in four districts without on-site laboratory services. Smear was conducted at centralized laboratory sites for all 12 districts [52].

3.4.2. Study population

Similar to the above study, the study population includes HIV positive patients presenting for HIV care and treatment at HIV clinics, all age group including children, male and female [21].

3.4.3. Sample size

All 6041 prospectively enrolled XPRES patients were included, 1816 in smear arm and 4225 in Xpert MTB/RIF arm [52].

However, for the purpose of this objective, the sample size estimation based on XPRES data is stated below. The sample size was estimated using data available from the XPRES parent study. This included interim data on proportion of unfavorable clinical outcome in the pre-Xpert MTB/RIF cohort (0.2, 8/41) and two sample ratio (0.44, pre-and Post-Xpert MTB/RIF sample). The estimated sample to detect a difference in odds ratio of 1.5, with 0.8 power

would be 1618 (1239 + 379). From XPRES study, 6041 (1816 smear arm and 4225 Xpert MTB/RIF arm) were enrolled and 2297 (712 smear arm and 1585 MTB/RIF arm) patients were presumptive TB.

Estimated sample sizes for a two-sample proportions test Pearson's chi-squared test.
Ho: p2 = p1 versus Ha: p2 not = p1

Alpha	Power	N	N1	N2	N-ratio	Delta	P1	P2	Odds-ratio
.05	.8	3084	2141	943	.44	1.3	2	.2453	1.3
.05	.8	1834	1273	561	.44	1.4	.2	.2593	1.4
.05	.8	1239	860	379	.44	1.5	.2	.2727	1.5
.05	.8	906	629	277	.44	1.6	.2	.2857	1.6
.05	.8	699	485	214	.44	1.7	.2	.2982	1.7
.05	.8	562	390	172	.44	1.8	.2	.3103	1.8
.05	.8	466	323	143	.44	1.9	.2	.322	1.9
.05	.8	394	273	121	.44	2	.2	.3333	2

STATA (STATA Corporation, statistical software: Release 13. College Station, Texas: StataCorp LP) was used to estimate the sample size (power two proportions .2, test [chi2] oratio [1.3(0.1)2.0] nratio [.44]).

3.4.4. Tuberculosis screening, diagnosis and treatment

At enrolment and each follow-up visit (i.e., at two weeks, then monthly for the first three months and then quarterly for the remaining follow-up period), adults were screened for TB using the standardised TB screening tool [56]. Children screened for weight loss or failure to thrive (no weight gain over 3 months), enlarged lymph nodes (more than 1 x 1 cm), cough for ≥ 2 weeks, fever for ≥ 2 weeks, fatigue/reduced playfulness ≥ 2 weeks and profuse night-sweats ≥ 2 weeks [13, 53].

3.4.5. Sputum collection

Patients who screened positive for at least one of the TB symptoms or signs were requested to provide four sputa; two were provided on the same day (Spot 1 and 2) and two on the

following day. Day 2 sputa included one early morning sputum collection at home (Morning sample) and another sample at the clinic (Spot 3). Patients in smear arm were enrolled in XPRES before Xpert MTB/RIF instrument implementation, therefore, Spot 1 and 3 were tested only with Ziehl–Nielson staining at the peripheral laboratory. However, if smear arm enrolees presented for a follow-up appointment after Xpert MTB/RIF instrument activation, and screened positive for TB during that follow-up visit, Spot 1 and 3 were tested by Xpert MTB/RIF at the peripheral laboratory or POC sites. For Xpert MTB/RIF arm enrolees, all Spot 1 and 3 samples were tested by Xpert MTB/RIF at either the peripheral laboratory or POC sites. Spot 2 and morning samples were submitted to the National TB Reference Laboratory for culture and drug susceptibility testing.

3.4.6. Data collection

TB screening, diagnosis, treatment and treatment outcome data were collected using standardized case report forms (CRF) between August 2012 and November 2014 [52] and were double entered into a Clindex database (Fortress Medical Systems, Minneapolis, MN, USA). Inconsistencies were identified through logic checks, and once identified were checked against the original CRF. Where possible, inconsistencies and missing data were corrected through review of patient charts.

3.4.7. Data Analysis

Data were analysed using STATA (STATA Corporation, 2015 statistical software: Release 14. College Station, Texas: StataCorp LP) [51] We used univariate and multiple logistic regression models; to describe and compare demographic and clinical characteristics of patients; and to account time to event we used Cox proportional hazard model to compare TB treatment outcome among TB patients diagnosed and treated using smear and Xpert MTB/RIF algorithm. All analyses were adjusted for within-facility correlation. *P* values of <0.05 were considered statistically significant.

3.5. Effect of sputum quality and volume on the yield of bacteriologically-confirmed TB

This is a sub-analysis using the data collected for the second sub-study (TB treatment outcome (section 3.2) and the detailed methodological approaches used were previously described (for publication see appendix 4) [57].

3.5.1. Setting

Similar to above studies, 13 GeneXpert instruments provided services for the 22 HIV care and treatment clinics, nine at centralized laboratory sites in eight districts and four POC at clinical care sites in four districts without on-site laboratory services. Smear was conducted at centralized laboratory sites for all 12 districts [52].

3.5.2. Study population

The study population includes HIV positive patients presenting for HIV care and treatment at HIV clinics, all age group including children, male and female [21].

3.5.3. Sample size

No additional sample size was calculated for this sub-study as this was a sub-analysis of the data collected for TB treatment outcome (sub-study two, section 3.2), where 6041 prospectively enrolled patients were included, 1816 in smear arm and 4225 in Xpert MTB/RIF arm.

3.5.4. Sputum collection and assessment of macroscopic sputum quality

TB screening and sputum collection was same as described above. Patients who screened positive for any of these TB symptoms or signs were requested to provide four sputa; two sputa provided on the same day (Spot 1 and 2) and another two on the following day (Morning sputum collected at home and Spot 3 collected at the clinic). In the main study, XPRES, after GeneXpert instrument implementation each Spot 1 and 3 sputum specimen were tested by both Xpert MTB/RIF and smear test was conducted at the peripheral laboratory. The focus of the sub-analysis was on patients who had at least one TB symptom and from whom sputum collected for both Xpert MTB/RIF and smear testing. Before GeneXpert instrument implemented each Spot 1 and 3 sputum specimen was tested only by smear at the peripheral laboratory as part of the main study.

Spot 2 and morning sputum were submitted to the National TB Reference Laboratory for culture. The culture result was needed for sensitivity analysis of the XPRES study, thus not included in this analysis.

Initially and throughout the study, study nurses received training on how to collect a high-quality sputum. The training included the importance of: (1) collecting a mucoid rather than salivary sample, (2) collecting the sample in a private but well-ventilated area outside the clinic; (3) use of a sputum collection job aid to guide patients through sputum production, and (4) use of observation and assistance (e.g. patting the back of the patient) to assist as they attempt to produce sputum. Guidelines were provided on sample storage and refrigeration while awaiting sample transportation to the peripheral laboratory.

At the beginning of the study, laboratorians at peripheral labs received refresher training on how to classify gross appearance of sputum. The training materials included a visual job aid, a coloured poster illustrating the different possible sputum appearance types and standard classification. Upon receiving a sample, laboratorians assessed the macroscopic appearance and volume of sputum samples by visualization and recorded results. Sputum appearance was graded into categories of salivary, mucoid, muco-purulent and blood-tinged or other. Sputum volume was measured in millilitres using a pre-calibrated sputum collection bottle as a reference. After grading the appearance and recording the volume of the sputum, the sputum samples were placed in a refrigerator until the time for smear or Xpert MTB/RIF testing.

3.5.5. Data collection

Data were collected using standardized case report forms between August 2012 and November 2014 [52] and double entered into a Clindex database (Fortress Medical Systems, Minneapolis, MN, USA). Inconsistencies and missing data were corrected through review of patient charts.

3.5.6. Data analysis

Data were analysed using STATA (STATA Corporation, 2015. statistical software: Release 14. College Station, TX: StataCorp LP) [51] to fit univariate and multiple logistic regression models; to describe and compare demographic and clinical characteristics of patients; and to compare patients with specimen specified sputum characteristic versus those without that characteristic [57]. We fit generalized-linear-mixed-models with appearance characteristics coded as salivary versus non-salivary, mucoid versus non-mucoid, and muco-purulent versus non-muco-purulent to estimate likelihood ratios (LR+, to report for fixed-effect) for sputum quality characteristics and the diagnostic yield of MTB testing (response analysed as an

outcome) and 95% confidence intervals. Log and binomial (log, bin) were used as a 'link function' and 'distribution (family)'. The models included a random effect for clinic to adjust for within-clinic correlation. These models first used to construct omnibus tests for all appearance indicators and all volume indicators for Xpert MTB/RIF and smear. If the omnibus test was significant, we proceeded to interpret the LR+ and confidence interval. If the omnibus test was not significant, we considered all pairwise comparisons non-significant. LR+s were defined as:

1) Prevalence of a given sputum characteristic in Xpert MTB/RIF MTB-positive samples divided by the prevalence of the same sputum characteristic in Xpert MTB/RIF MTB negative samples.
2) Prevalence of a given sputum characteristic in AFB smear positive of sputum samples divided by the prevalence of the same sputum characteristic in smear negative samples.

P values of <0.05 were considered statistically significant [57].

3.6. Higher-than-expected prevalence of non-tuberculous mycobacteria in HIV setting in Botswana: Implications for diagnostic algorithms using Xpert MTB/RIF assay.

The methodological approaches described hereunder are per the attached (appendix 4) published paper [58].

3.6.1. Setting

Culture, DST and LPA were conducted at the national TB reference laboratory at Gaborone, capital city. The rest is similar to above studies, 13 GeneXpert instruments provided services for the 22 HIV care and treatment clinics, nine at centralized laboratory sites in eight districts and four POC at clinical care sites in four districts without on-site laboratory services. Smear was at centralized laboratory sites for all 12 districts [58].

3.6.2. Study population

Similar to the above three studies, the study population includes HIV positive patients presenting for HIV care and treatment at HIV clinics, all age group including children, male and female [21].

3.6.3. Sample size

All of the 6041 prospectively enrolled XPRES patients were included and with that, there was adequate number of patients.

However, for the purpose of this objective the estimated sample size was calculated based on the preliminary NTM positive proportion of sputum cultures under the XPRES study. At the time of the PhD study design 55% of main study (XPRES) cultures at the national TB reference laboratory were NTM positive-culture. We used the formula below, to estimate the sample size for a single proportion.

$n = (z)^2 * p * (1-p)/W^2 = (2*1.96)^2 * p * (1-p)/W^2 = 15.4 * p * (1-p)/W^2$

- where n = the required sample size
- z = 1.96 for 95% confidence interval
- p = the expected proportion - here 0.55
- W = width of the 95% confidence interval - here 0.05 (52.5% - 57.5% = 5% = 0.05).

$n = 3.84 * 0.55 * (1- 0.55/ (0.05)^2 = 15.4*0.55* (0.45/0.0025) =$
$3.84 *0.55*180=380.$

In XPRES, a total of 1940 PLHIV presented with TB symptoms and were able to submit at least one sputum specimen for culture testing that was more than the estimated sample size for Non-tuberculous mycobacterium (NTM) proportion.

3.6.4. Screening for TB and sputum collection

All HIV infected patients presenting for HIV care and treatment services at 22 clinical sites were recruited and offered screening for TB from August 2012 through November 2014. For

patients who screened positive for at least one of the four TB symptoms (any duration of cough, fever, night sweats and weight loss), demographic data [CD4 count, body mass index (BMI) and history of TB] were collected. Patients were asked to submit four sputum samples: 2 spots specimens (1 and 2) on the day of screening and 1 morning and 1 spot (spot 3) on the following day [58].

Spot specimens one and three were tested by Xpert MTB/RIF and Ziehl Neelsen (ZN) smear at the peripheral laboratory. Morning and spot two specimens transported to the National TB Reference Laboratory (NTRL) for culture and drug susceptibility testing (DST) [58]. Similar methods reported by Aliyu *et al* was used to process and test at NTRL [48]. Sputum samples were treated with BD Mycoprep (Beckton Dickinson, Sparks, Maryland, United States of America (USA)) which consists of 1% N-acetyl-L-cysteine (NALC), 4% sodium hydroxide and 2.9% sodium citrate then incubated in the automated BACTEC MGIT 960 instrument (Becton Dickinson, Sparks, Maryland, USA) [48, 58].

Samples that failed to show any growth after 42 days of incubation in the MGIT 960 were classified as negative based on the manufacturer protocol [48, 58]. Samples with positive-growth were inoculated on blood agar to check for non-mycobacterial contamination. Then, a ZN smear performed to check for the presence of Acid-Fast Bacilli (AFB) [48, 58].

Cultures with positive-growth in the MGIT 960 and presence of AFB by ZN were tested with a rapid TB immunochromatographic assay (SD-Bioline Ag MPT64 RapidTM assay, Standard Diagnostics, Kyonggi-do, Korea) to discriminate between NTM and MTBC [48, 58]. Cultures with positive-growth in the MGIT 960 and presence of AFB but that were negative for MTBC using the SD-Bioline assay were sub-cultured on Lowenstein Jensen (LJ) media [48, 58]. Those that subsequently grew on LJ medium were considered to be presumptive NTMs and were characterized to species level with LPA (GenoType CM and AS assays, Hain Lifescience, Nehren, Germany) according to manufacturer recommendations [48, 58].

To describe the specificity of Xpert MTB/RIF in our setting, all patient who had a culture-positive isolate identified as NTM were tested with Xpert MTB/RIF retrospectively. To describe the geographic distribution of we recorded NTN positive cultures by origin of samples in the 12 districts included in the study [48, 58].

3.6.5. Data collection

Similar to the above study, data were collected using standardized case report forms between August 2012 and November 2014 [52] and double entered into a Clindex database (Fortress Medical Systems, Minneapolis, MN, USA). Inconsistencies and missing data were corrected through review of patient charts.

3.6.6. Data analysis

We used STATA (STATA Corporation, 2015 statistical software: Release 14. College Station, Texas: StataCorp LP) [51] to fit univariate and multiple logistic regression models to compare demographic and clinical characteristics between patients with MTBC and NTM, adjusted for within-clinic correlation. *P* value of <0.05 was considered statistically significant. We also used descriptive comparison of geographic mapping of origin of NTMs among the 12 districts included in the study [58].

3.7. TB treatment-outcomes among patients diagnosed using Xpert MTB/RIF: A systematic review and meta-analysis Search strategy

The methodological approaches described hereunder are per the attached (appendix 4) published paper [59].

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [60]. The systematic review and meta-analysis is registered at PROSPERO, http://www.crd.york.ac.uk/PROSPERO, number CRD42016050625.

Since Xpert MTB/RIF is a new TB diagnostic technology in use, we searched PubMed and SCOPUS for all relevant articles published between January 2000 and December 2016 [59]. A sensitive search strategy was used with Xpert MTB/RIF as the key heading term used in combination with potentially relevant key factors: Xpert MTB/RIF AND "tuberculosis"; or “impact”; or “effect”; or “treatment outcome”; or “time to diagnosis”; or “time to treatment”; or “empiric treatment”: or “loss-to-follow-up”; or “mortality”; or “morbidity” [59].

We also reviewed abstract books from the Union World Conference on Lung Health from 2012 to 2016 for studies that may have been completed but not published yet [59]. In

addition, we reviewed the reference lists of the primary studies to include additional references that might have been missed with our primary search strategy [59].

3.7.1. Review of studies

Studies designed as cohort studies or randomized clinical trials comparing the diagnosis and/or treatment outcomes of TB patients diagnosed by Xpert MTB/RIF versus smear were potentially eligible to be included in the review [59]. Citations with patient level treatment outcome, favorable and unfavorable, comparing MTB/RIF and smear were eligible for inclusion in the meta-analysis [59]. The target study population included patients of any age or sex who presented with TB symptoms at any health care level. The inclusion criteria were studies reporting on: (1) treatment outcomes for drug-sensitive TB patients, diagnosed using Xpert MTB/RIF compared to those diagnosed by smear, (2) the effect of Xpert MTB/RIF on time to diagnosis, time to treatment, empiric treatment or mortality. The exclusion criteria were: (1) studies reporting on treatment outcomes for drug-resistant TB, (2) studies not reporting on patient-level treatment outcomes or whose reporting was from modelling studies or population level impact [59].

The two reviewers (TA and RB) independently reviewed study titles and abstracts to determine eligibility. All studies that met the inclusion criteria received full text reviews and any differences resolved by consensus [59].

3.7.2. Data extraction and statistical analysis

For studies fulfilling the inclusion criteria, two independent reviewers selected eligible citations and compiled data on a pre-piloted Excel spreadsheet [59].

The following information were collected, as available: Article author and title, year of publication, study setting, population (HIV-infected and/or -uninfected), participant demographics and baseline characteristics, details of intervention and control conditions, study methodology, treatment outcomes, time to diagnosis, time to treatment, diagnostic test (smear and Xpert MTB/RIF), incremental value (additional TB cases diagnosed) and description of empiric treatment practices [59].

Favorable TB treatment outcomes were defined as cured or completed treatment. Unfavorable treatment outcomes were defined as failed, death or loss-to-follow-up [59]. Empiric treatment was defined as initiation of treatment in the absence of a bacteriologically confirmed diagnosis [59]. Diagnostic impact (decreased time to diagnosis, increased number of TB cases diagnoses and increased bacteriologically-confirmed TB), therapeutic impacts (decreased time to treatment, decreased loss-to-follow-up and reduced empirical treatment) and patient-level outcomes (improved treatment outcome including mortality) were described per Schumacher *et al* framework [29].

The abstracted data were aggregated and summarized as favorable and unfavorable outcomes for patients diagnosed and treated using the smear- and Xpert MTB/RIF-based algorithms [59]. Pooled analysis was conducted using a Mantel-Haenszel method with a fixed-effect model and a forest plot created using STATA version 14.0 (STATA Corporation, 2015 statistical software: Release 14. College Station, Texas: StataCorp LP) [51]. The statistical test, I square (I^2), was applied to measure heterogeneity. To support the results from the pooled analysis, a narrative analysis of articles that did not meet the criteria for meta-analysis was produced to identify factors potentially affecting treatment outcomes (time to diagnosis and time to treatment, empiric treatment and bacteriologically-confirmed TB) [59].

3.7.3. Quality assessment

While scales such as the Detsky *et al* scale can be useful, many authors consider the scoring schemes to be imprecise and cut-off points can be arbitrary and for this reason, we have not used any scale or scoring system [61, 62]. We applied a set of strict inclusion and exclusion criteria to identify as many comparable studies as possible. For consistency, and to ensure high quality of data two authors (TA and RB) independently reviewed methods used in potentially eligible articles and abstracts [59]. The included citations provided information on how patient-level treatment outcomes measured and reported results using either risk-ratio (RR), 95% confidence interval (CI) or *P values*. Following review, any concern or uncertainty was resolved by consensus [59].

3.8. Ethics

The PhD protocol is a study embedded under XPRES clinical trial. The XPRES protocol and data collected therein was approved by the U.S. regulatory agency, Centers for Disease Control and Prevention (CDC) institutional review board (IRB), (approval date 15 April 2014), and the Botswana health research and development committee, (approval date 2 June 2014). All patients at study sites were enrolled in the study following the IRB-approved, written, informed consent (for adults) and assent (for children under 18 years old). In addition, the PhD protocol developed on a broader context to address the three doctoral research questions was also approved by WITS Human Research Ethics Committee for approval as part of the PhD process (12 Jul 2017) [52, 59].

CHAPTER 4 RESULTS

The summary results from the doctoral study is presented in the order presented in Table 1 beginning from Xpert MTB/RIF implementation and performance followed by diagnostic and therapeutic impact to patient level clinical outcome, barriers to success to clinical impact of Xpert MTB/RIF (sputum quality and volume, empiric treatment especially on patients with NTM). Lastly, a systematic review and meta-analysis of treatment outcome, diagnostic and therapeutic impact of Xpert MTB/RIF are presented as part of the doctoral study. The detailed results from each study is presented in the respective publication/manuscripts attached herewith (appendix 4).

Table 1. Summary of manuscripts completed/published

Study	Title	Progress
1	Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic trial in Botswana	Published, PLoS One Aug 2017
2	Tuberculosis treatment outcomes among people living with HIV diagnosed by Xpert MTB/RIF versus smear in Botswana	Manuscript submitted to PLoS One, Jun 2019, under review
3	The Effect of sputum quality and volume on the yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear	Published, PAMJ, Jun 2019
4	Higher-than-expected prevalence of non-tuberculous mycobacteria in HIV setting in Botswana: Implications for diagnostic algorithms using Xpert MTB/RIF assay	Published, PLoS One Dec 2017
4 (a)	Treatment outcomes among people living with HIV with non-tuberculosis mycobacteria versus Mycobacterium tuberculosis in Botswana	Published abstract, IJTLD, Oct 2017
5	Treatment-outcomes, diagnostic and therapeutic impact: Xpert MTB/RIF versus smear systematic review and meta-analysis	Published, IJTLD, Jan 2019

4.1. Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic trial in Botswana (study one)

Summary of result from study one presents the overall performance (valid/failed/invalid results) of Xpert MTB/RIF and in comparison with POC sites and laboratory site. In addition, the effect of environmental temperature, electric power supply and regularity of calibration presented. All results presented under 4.1 are per the published paper from this PhD [52].

In this doctoral analysis, we reported that 254 clinical personnel at study sites trained at study initiation to implement the Xpert-based TB diagnostic algorithms using the daylong training curriculum [52]. Of those trained 77 were medical officers, 126 were registered nurses, 28 were laboratorians and 23 were staff of other cadres [52]. Simultaneously with GeneXpert installation GeneXpert operators at each study site received three-day initial trainings. Forty-six GeneXpert operators were trained, among whom 17 (37%) were nurses at POC sites. During the supervisory visits, an additional 32 GeneXpert operators, including 12 nurses,

were trained per request from the implementing sites, resulting in a total of 78 who received the three-day standard MoHW-recommended training [52]. During supervisory visits, we found that 21 staff members (20 laboratorians and one nurse) who did not receive the standard three-day training were conducting Xpert MTB/RIF testing [52].

During our supervisory visits, we observed and documented that: (1) every three months laboratorians were rotating from one laboratory section to another and every two years from one laboratory to another, and this led to Xpert MTB/RIF testing sites with qualified staff constraints; (2) at two sites, out of the laboratorians conducting Xpert MTB/RIF, more than half received only in-house training; (3) operators did not always adhere to Standard operating procedures (SOP), with frequently observed mistakes including: variable sample and reagent mixing time, inconsistent daily, weekly and monthly GeneXpert maintenance, and inconsistent daily room temperature monitoring [52]. Reminders and demonstrations by supervising study staff were necessary to improve adherence to SOPs; (4) GeneXpert operation at the four POC sites was interrupted more than once because a nurse was not available for testing. Reasons encountered included: (a) heavy nursing workloads and short staffing in busy clinics making it difficult for the nurses to break away to perform the test; (b) inadequate coverage during night shift rotations and leave (night off the following day) schedules; and (c) reluctance to operate the GeneXpert because it was initially perceived as a non-nursing duty [52].

Xpert MTB/RIF test results

A total of 3630 Xpert MTB/RIF tests were performed over 276 instrument-months of operation. Of these, 3102 (85%) were successful. Among all tests conducted, 447 (12%) were positive for MTBC (Table 2), ranging from 1-23% depending on the testing site [52]. Among all 3102 successful tests, MTBC was positive in 14%. Among samples that were positive for MTBC, the proportion with RIF-resistance was 8% (36) and 3% (15) were indeterminate [52].

Table 2. Xpert MTB/RIF implementation of results at laboratory and POC in Botswana, October 2012 to November 2014.

Gene Xpert site	Tested N (%)	MTBC‡ Detected		Rifampicin Resistance						Unsuccessful test		Type of unsuccessful test						Valid test	MTBC w/o unsuccessful test
		N	%	Detected		Indeterminate		Not Detected		n	%	Error		Invalid		No result			
				n	%	n	%	n	%			n	%	n	%	n	%	n	%
ATH **	197 (5)	26	13%	3	12%	2	8%	21	92%	29	15%	25	86%	2	7%	2	7%	168	15%
AWC **	641 (18)	78	12%	10	13%	0	0%	68	87%	115	18%	91	79%	17	15%	7	6%	526	15%
BK8 **	560 (15)	67	12%	7	10%	2	3%	58	87%	72	13%	34	47%	33	46%	5	7%	488	14%
BOB **	200 (6)	20	10%	2	10%	2	10%	16	80%	16	8%	13	81%	3	19%	0	0%	184	11%
DRM **	150 (4)	11	7%	1	9%	2	18%	8	73%	12	8%	9	75%	3	25%	0	0%	138	8%
EXT*	161 (4)	2	1%	0	0%	0	0%	2	100%	14	9%	9	64%	4	29%	1	7%	147	1%
GAN **	166 (5)	35	21%	2	6%	2	6%	31	89%	17	10%	12	71%	4	24%	1	6%	149	23%
KAD *	259 (7)	32	12%	2	6%	2	6%	28	88%	38	15%	16	42%	22	58%	0	0%	221	14%
LMH **	292 (8)	21	7%	2	10%	0	0%	19	90%	45	15%	36	80%	1	2%	8	18%	247	9%
MCC*	397 (11)	67	17%	5	7%	0	0%	62	93%	79	20%	47	59%	14	18%	18	23%	318	21%
NKO *	265 (7)	28	11%	1	4%	0	0%	27	96%	50	19%	44	88%	3	6%	3	6%	215	13%
NRH **	121 (3)	28	23%	1	4%	0	0%	27	93%	19	16%	16	84%	0	0%	3	16%	102	27%
SDA **	221 (6)	32	14%	0	0%	3	9%	29	91%	22	10%	9	41%	13	59%	0	0%	199	16%
All sites	3630 (100%)	447	12%	36	8.1%	15	3.4%	396	89.0%	528	14.5%	361	68.4%	119	22.5%	48	9.1%	3102	14.4%

*** Point of care (POC) ** Centralized laboratory**

‡ **MTBC =** *Mycobacterium tuberculosis* complex

Athlon Hospital = ATH, Area W Clinic = AWC, Block 8 Clinic = BK8, Bobonong Primary Hospital = BOB, Deborah Retief Memorial Hospital = DRM, Ext 3 Clinic = EXT, Gantsi Hospital = GAN, Kadimo Clinic = KAD, Letsholathebe II Memorial Hospital = LMH, MCC Clinic = MCC, Nkoyaphiri Clinic = NKO, Nyangabgwe Referral Hospital = NRH and SDA Hospital = SDA

Source: Agizew T et al*. Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic, stepped-wedge trial in Botswana. PLoS One 2017 17;12(8):e0183237.*

Incidence and type of unsuccessful (failed) Xpert MTB/RIF **tests**

A total of 528 (15%, 95% CI, 12-17%) Xpert MTB/RIF test results recorded as unsuccessful and the cumulative unsuccessful test incidence varied from 8-20% across sites (Table 2) [52]. Among all tests conducted, the majority of unsuccessful tests were errors, 361/3630 (10%, 95% CI, 7-13%). Invalid results made up 119/3630 (3%, 95% CI, 2-5%) of all tests conducted and no result were obtained in 48/3630 (1%, 95% CI, 1-3%). The error, invalid, and no result incidence percentages varied by site, ranging from 4–17%, 0–8% and 0–5%, respectively (Table 2) [52]. Cumulative incidence of errors was higher than the manufacturer predicted error rate of 5% in 12 out of the 13 sites [63].

Incidence of unsuccessful (failed) Xpert MTB/RIF **test at POC and centralized laboratory sites**

When results were stratified by type of site, unsuccessful test incidence was higher at POC (181/1082, 17%, (95% CI, 11-25%) versus centralized laboratory (347/2548, 14%, 95% CI, 11-17%), though the difference was not statistically significant (*p=0.14*) [52].

Similarly, the error, invalid, and no result rate were not statistically significant between tests performed at POC versus centralized laboratory sites (Table 3) [52].

Table 3. Type of unsuccessful tests by Xpert MTB/RIF testing sites

	POC* N=1082 (100%)			**Centralized laboratory** N=2548 (100%)			
Type of unsuccessful test	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	***p value***
Error	116	11%	5-21%	245	10%	6-14%	0.87
Invalid	43	4%	1-13%	76	3%	2-6%	0.08
No result	22	2%	0-13%	26	1%	1-2%	0.31

* POC = Point-of-Care

***Source: Agizew T et al**. Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: experience gained from a pragmatic, stepped-wedge trial in Botswana. PLoS One 2017 17;12(8):e0183237.*

Incidence of error by type

The error codes were recorded by category based on the most common underlying causes of unsuccessful tests: sample processing, power supply, cartridge/module related, temperature-related, other GeneXpert related [6]. Overall, 385 total errors codes were recorded from 361 error results; for 24 tests more than one type of error code was recorded [52]. The most common type of errors were related to sample processing (codes 2008, 5006 and 5007) (55%), followed by power supply (code 2127) (20%), cartridge/module-related (code 2032 and 5011) (14%), temperature-related (codes 1001, 1002, and 2014) (5%) and other GeneXpert errors (code 2034, 2035 and 1006) (6%) [19, 52]. The type of error did not differ significantly between POC and centralized laboratory sites.

Comparing incidence of error between G3 and G4 Cartridges

The difference in error proportion was not statistically significant for tests using G3 and G4 cartridges, (41/432, 9% versus 320/3198, 10%, *p=0.86).* However, the proportion of errors of type 5011 (loss of tube pressure) & 2032 (ultrasonic horn could not be tuned properly) was significantly higher with G3 (14/41, 34%) than with G4 (40/344, 12%), *p=0.002* [52].

Evaluating association between training level and incidence of unsuccessful test or incidence of "error"

Twenty-one percent (21/99) of GeneXpert operators were trained in-house. We examined the effect of in-house operator training on incidence of unsuccessful tests and error, categorizing percentages as 0-10, >10-20, >20-30, >30-40, >40-50 and > 50. The omnibus (overall) tests were not significant for either unsuccessful tests *(p=0.06)* or for error *(p=0.24)*, so we did not do pairwise comparisons between training levels [52].

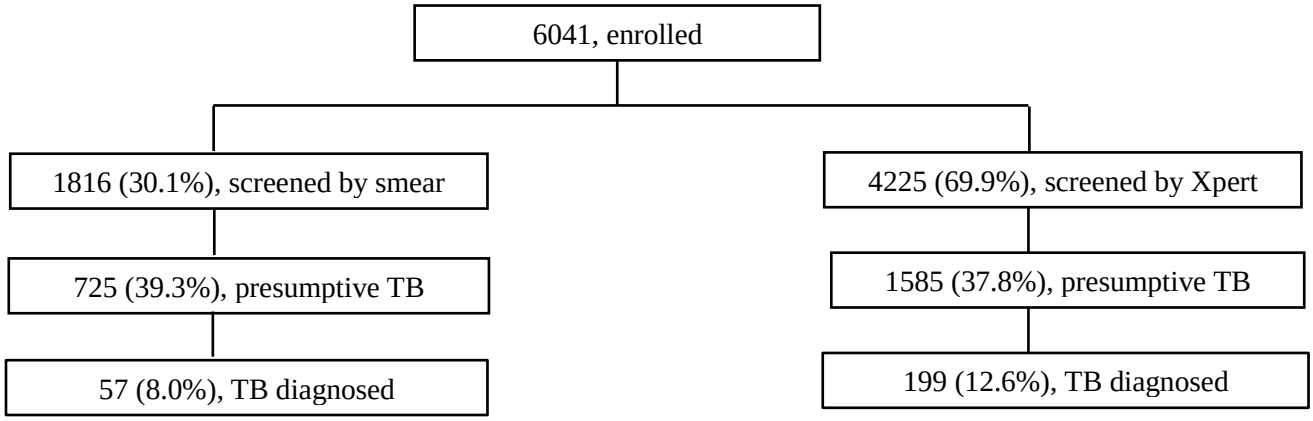
4.2. Tuberculosis treatment outcomes among people living with HIV diagnosed by Xpert MTB/RIF versus smear in Botswana (study two)

Summary of result from study two presents the diagnostic and therapeutic impact of Xpert MTB/RIF on patient-level treatment outcome in comparison with smear. All results presented

under 4.2 are as per the manuscript (Tuberculosis treatment outcomes among people living with HIV diagnosed by Xpert MTB/RIF versus smear in Botswana) attached in Appendix 4.

In our finding among 6041 patients, 1816 patients were enrolled and screened using smear (smear arm) and 4225 screened using Xpert MTB/RIF (Xpert MTB/RIF arm). Upon initial consultation and follow up, 2297 (712 among patients in smear arm and 1585 in Xpert MTB/RIF arm) were presumptive TB (screened positive for one of four TB symptoms, cough, fever, night sweat, weight loss). A total of 256 (199 per 2985 person-years and 57 per 1582 person-years of follow-up, in Xpert and smear arm, respectively, adjusted incidence rate ratio, 9.07, 95% confidence interval (CI) 4.70-17.48, *P<0.001*) were diagnosed with and treated for drug-sensitive TB. The rate of TB among presumptive TB in Xpert and smear arm was 12.6% (199/1585) versus 8.0% (57/712), OR 1.65, 95% CI 1.21-2.24, *p = 0.001* (Fig. 4).

Fig. 4. Enrolment, screening by smear, by Xpert MTB/RIF and TB diagnosis in Botswana



Demographic characteristics among patients with and without TB:

Patients with TB (256; Table 1) were more likely to have CD4 count less than 200 cells/mm^3 (aOR, 2.16, 95% CI: 1.72-2.72, *p <0.001*) and BMI < 18.5 (aOR, 2.41, 95% CI: 1.80-3.23, *p <0.001*) ,and were less likely to be younger (age < 35 years), use of alcohol , aOR, 0.68, 95% CI: 0.46-0.99, *p = 0.047*) participants with no TB (5785; Table 1). There was no statistically significant difference of gender, previous history of TB treatment, current or history of smoking and miner between the participants, with or without TB (Table 4).

Table 4. Characteristics of HIV patients with and without TB screened in HIV clinics in Botswana

	Patients with TB			Patients without TB					
Characteristics	**N**	**n**	**(%)**	**N**	**n**	**(%)**	**aOR ***	**95% CI****	***p value***
Age < 35	256	108	42.2%	5784	3186	55.1%	0.73	0.55-0.97	0.034
Gender, female	256	128	50.0%	5785	3902	67.5%	0.76	0.52-1.12	0.16
CD4 count <200 cells/ mm^3	252	158	62.7%	5669	2162	38.1%	2.16	1.72-2.72	<0.001
BMI <18.5	256	104	40.6%	5785	1116	19.3%	2.41	1.80-3.23	<0.001
Previous TB	255	33	12.9%	5778	613	10.6%	0.84	0.56-1.27	0.40
Smoking***	256	74	28.9%	5769	1088	18.9%	1.35	0.96-1.89	0.08
Alcohol use	256	55	21.5%	5770	1266	21.9%	0.68	0.46-0.99	0.047
Miner	256	25	9.8%	5770	278	4.8%	1.58	0.90-2.79	0.11

***** aOR=adjusted Odds Ratio
****** CI=Confidence interval
******* Current or x-smoker

TB treatment outcomes

By the end of the study, TB treatment outcomes as favorable or unfavorable were available for 203/256 (79.3%); 46 were under smear and 157 Xpert arms. Fifty-three of the 256 TB patients were transferred out or were not evaluated. Although not statistically significant, persons with a TB diagnosis informed by sputum-smear microscopy were more likely to have unfavorable treatment outcomes as compared to persons with TB diagnosis informed by Xpert MTB/RIF (aHR:1.40; 95% CI: 0.75-2.26, $p = 0.27$) (Table 5).

Table 5. Tuberculosis treatment outcome among people living with HIV screened using smear and Xpert MRB/RIF in Botswana

Treatment outcomes	TB patients screened by smear n=57	TB patients screened Xpert MTB/RIF n=199	Adjusted hazard ratio*	95% CI*
Unfavorable outcome[¥]	10	21	1.40	0.75-2.26
Favorable outcome[£]	36	136		
Sub Total	**46 (80.7)**	**157 (78.9%)**		
Transferred out or not evaluated	11 (19.3%)	42 (21.1%)		

* adjusted for inter-facility difference, *P value = 0.27*

[¥] death, default, failure

[£] cured/completed

Factors affecting treatment outcomes

Placement of Xpert MTB/RIF POC versus laboratory

Among 157 TB patients diagnosed using Xpert MTB/RIF and with treatment outcome, 48 and 109 were from POC and laboratory sites, respectively. Although was not statistically significant, unfavorable treatment outcome was higher at POC, compared to laboratory, 7/48 (14.6%) versus 14/109 (12.8%), respectively, aOR, 1.16, 95% CI: 0.37-3.66, *p=0.80.*

Time to treatment

The median number of days (and Interquartile Range, IQR) from sputum collection to TB treatment among patients in smear and in Xpert MTB/RIF arm were 21 (3-47) and 6 (2-16), respectively, *p*=0.008, Wilcoxon-rank-sum (Mann-Whitney) test. Time to treatment in the Xpert MTB/RIF arm was shorter, and unfavorable outcome was higher among smear arm than Xpert MTB/RIF, though the difference was not statistically significant, 4/35 (11.4%) vs 10/121 (8.3%), aOR 1.43, 95% CI: 0.63-3.27, *p=0.37.*

Empiric treatment

Empiric TB treatment in smear arm was more than twice higher than the Xpert MTB/RIF arm, 39/57 (68.4%) versus 97/199 (48.7%), respectively, aOR, 2.28, 95% CI: 1.24-4.20, *p=0.011*), respectively.

Microbiologically-confirmed TB

Patients in Xpert arm who submitted at least one sputum for testing were more likely to be diagnosed with microbiologically-confirmed TB than those patients in a smear arm, 102/173 (59.0%) versus 18/43 (41.9%), respectively, aOR, 2.00, 95% CI: 1.01-3.96, *p=0.048*).

Loss-to-follow-up

Lost to follow up between the two arms, smear and Xpert MTB/RIF: the difference was not statistically significant with 1/46 (2.2%) versus 4/157 (2.5%), aOR, 1.18, 95% CI: 0.09-14.76, *p=0.90*), respectively.

4.3. The effect of sputum quality and volume on the yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear (study three).

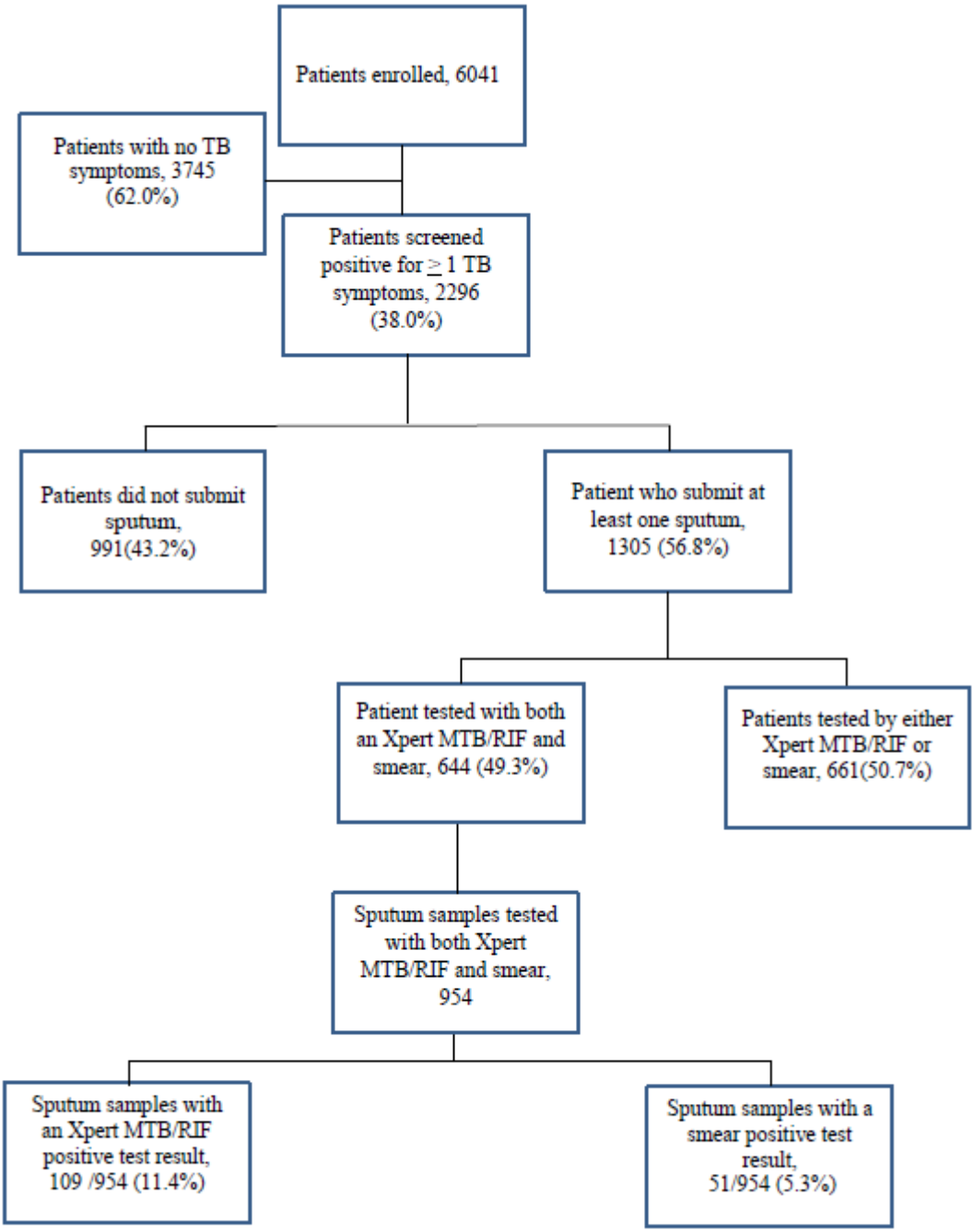
Summary of result from study three presented the effect of appearance (quality) and volume (quantity) of sputum on Xpert MTB/RIF test result. The results presented under 4.3 are per the published paper from the PhD [57].

The doctoral study provided a unique opportunity to evaluate the effect of sputum quality and volume on the yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear. Among 6,041 patients enrolled 2,296 (38.0%) screened positive for TB symptoms at enrolment or follow-up. Of these, 1,305 (56.8%) patients submitted ≥1 sputum sample and 991 (43.2%) did not have an available sputum sample for testing. There were no significant differences on demographic characteristics among those who did not submit sputum and those who submitted sputum, age <35 was 47% (438/937) versus 42% (538/1278), aOR 1.21, 95% CI: 0.99-1.46, *p=0.054*; and gender, female, 60% (564/937) versus 57% (724/1278), aOR 1.16, 95% CI: 0.95-

1.42, *p=0.14)* and CD4 counts, 52% (481/925) versus 49% (619/1,264), aOR 1.13, 95% CI: 0.90-1.42, *p= 0.28*. The difference was observed on symptom of cough, those who did not submit sputum were less likely to have symptom of cough versus who submitted sputum, 30% (278/937) versus 65% (832/1277), aOR 0.23, 95% CI: 0.17-0.30, p < 0.001. Of the 1,305, 644 (49.3%) patients had both smear and Xpert MTB/RIF test results and 661 (50.7%) were only tested by either Xpert MTB/RIF or smear and not both (Fig. 5) [57].

Combined 991and 661, the 1,652 patients will be referred to as patients that "did not receive sputum testing by both Xpert MTB/RIF and smear'. We compared the demographic and clinical characteristics of patients (1,652) that did not receive sputum testing by both Xpert MTB/RIF and smear, and those patients (644) tested by both methods. Overall, there was no statistically significant difference in the two populations in terms of age, gender, CD4 status, history of previous TB treatment and presenting symptoms. Patients who did not receive sputum testing by both Xpert MTB/RIF and smear presented less frequently with cough (45.8% versus 62.3%, aOR 0.50, 95% CI: 0.36-0.70, *p<0.001*) and smoking (24.3% versus 30.9%, aOR 0.67, 95% CI: 0.50-0.89, *p=0.008*) than those tested by both smear and Xpert MTB/RIF [57].

Fig. 5. Patients with at least one sputum culture results by mycobacterium species August 2012 – November 2014



Source: Zimba and Agizew et al: The effect of sputum quality and volume on the yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear. Pan African Medical Journal. 2019;33:110. doi:10.11604/pamj.2019.33.110.15319.

Predictive value of sputum appearance and volume on bacteriologically-confirmed diagnostic yield

From 644 patients, 954 sputum samples were tested by both Xpert MTB/RIF and smear; 43.3% (413) were salivary, 14.4% (137) were mucoid, 40.5% (386) were muco-purulent, and 1.0% (10) was blood tinged. The percentage of patients with salivary sputum ranged from 24-70% among study sites. The diagnostic yield of MTB detected by Xpert MTB/RIF was more than two-fold higher than that of smear (11.4% versus 5.3%), *p<0.001*). For any category of sputum quality measures (gross appearance or volume), the diagnostic yield of bacteriologically-confirmed diagnostic yield was approximately two-fold higher when tested by Xpert MTB/RIF compared to smear (Table 6) [57].

Table 6. Sputum characteristics from PLHIV with presumptive TB

Characteristics	n	Xpert MTB/RIF positive (%)	LR+ * (95% CI**)	Smear Positive (%)	LR+ (95% CI)
Gross appearance[1]					
Salivary	413	54 (13.1)	1.17 (1.00-1.37)#	27 (6.5)	1.24 (0.82-1.86)
Mucoid	137	14 (10.2)	0.88 (0.56-1.38)	5 (3.7)	0.67 (0.27-1.70)
Muco-purulent	386	38 (9.8)	0.85 (0.67-1.06)	19 (4.9)	0.92 (0.62-1.36)
Blood-tinged	10	0 (0.0)	n/a	0 (0.0)	n/a
Other	8	3 (37.5)	n/a	0 (0.0)	n/a
Volume[2]					
<1ml	2	0 (0.0)	n/a	0 (0.0)	n/a
1ml to < 2ml	272	20 (7.4)	0.62 (0.44-0.87)	7(2.3)	0.47 (0.22-1.01)
2 ml to < 3ml	330	46 (13.9)	1.26 (1.05-1.50)	25 (7.6)	1.45 (1.01-2.08)
3 ml to < 4ml	62	9 (14.5)	1.32 (0.65-2.68)	5 (8.1)	1.55 (0.75-3.20)
4 ml to < 5ml	28	2 (7.1)	0.60 (0.10-3.73)	0 (0.0)	n/a
>= 5ml	260	32 (12.3)	1.09 (0.72-1.64)	14 (5.4)	1.01 (0.62-1.63)
Total	954	109 (11.4%)§	-	51(5.3%)	-

***** Likelihood ratio positive for bacteriologically-positive sputum.
** CI: Confidence interval.
# Lower confidence limit is rounded up from 0.995
§ Difference in proportions, bacteriologically-positive, between Xpert MTB/RIF and smear is significant. *p<0.001*
[1]Omnibus tests for appearance (Xpert MTB/RIF and smear) not significant, *p>0.05.*
[2]Omnibus test for volume was significant (*p<0.05*) for Xpert MTB/RIF, but was non-significant for smear.

Source: Zimba and Agizew et al: The effect of sputum quality and volume on the yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear. Pan African Medical Journal. 2019;33:110. doi:10.11604/pamj.2019.33.110.15319.

Sputum appearance was not predictive of bacteriologically-positive sputum by either Xpert MTB/RIF or smear (Omnibus test $p > 0.05$). The omnibus test for sputum volume for Xpert MTB/RIF testing was significant ($p < 0.05$) and sputum volume of 2ml to < 3ml was predictive of a positive sputum Xpert MTB/RIF result, LR+=1.26 (95% CI: 1.05–1.50). On the other hand, sputum volume 1ml to <2ml was less predictive of a positive Xpert MTB/RIF result, LR+=0.62 (95% CI: 0.44-0.87). The proportion of bacteriologically-confirmed diagnostic yield showed no improvement by either Xpert MTB/RIF or smear as sputum quality improved (Table 6) [57].

4.4. Higher-than-expected prevalence of non-tuberculous mycobacteria in HIV setting in Botswana: Implications for diagnostic algorithms using Xpert MTB/RIF assay (study four)

Summary of result from study four presented the prevalence of NTM in Botswana measured from the culture reports and speciation by geographic distribution in Botswana. All results presented under 4.4 were per published paper [58].

Using XPRES study cohort, from August 2012 to November 2014, 16, 259 PLHIV enrolled and 10, 213 screened for TB symptoms and for the remaining 6046 only data abstraction done due to amendment to main study (XPRES) data collection procedure [58]. Among patients screened for TB symptoms, 3068 screened positive for $\geq$ 1 TB symptom. Of these, 1940 patients were able to submit sputum specimens for both culture and Xpert MTB/RIF testing. The median age was 37 (interquartile range, IQR 31-45), 1147/1940 (59%) were female, and the median baseline CD4 count was 249 cells/mm^3 (IQR 127-340) [58]. Among 1940, 427 (22%) had ≥1 specimen with a positive-culture result by MGIT 960 [58]. Of these 247 and 180 identified phenotypically as having isolates with NTM and MTBC, respectively. Nineteen of the 247 patients initially identified as NTM later excluded as NTM based on additional genotypic testing with LPA [58]. One of the patients with NTM also identified with additional MTBC after genotypic testing. Of the remaining 408 patients with identified species 228 (56%, 95% confidence interval (CI), 46-66%) were NTM and 180 (44%, 95% CI, 39-49%) were

MTBC [58]. The proportion of patients who had two culture positives for NTM and MTBC, respectively, were 3.5% (8/228) versus 16.7% (30/180), OR=0.18, 95% CI, 0.08-0.40, *p<0.001* [58].

Demographic characteristics, CD4 count, BMI, history of TB and presenting clinical features were not statistically significant among patients with culture-positive NTM and MTBC, except age ≥50 that was higher among patients with culture-positive NTM (19.7% versus 12.2%, aOR, 1.67, 95% CI: 1.02-2.74, *p <0.044*) [58]. Anemia (Haemoglobin, Hgb, <10mg/dl) was less common among patients with culture-positive NTM culture than culture-positive MTBC (15.7% versus 31.4%, aOR, 0.26, 95% CI: 0.09-0.73, *p = 0.015)* [58]. Patients with culture-positive NTM were significantly less likely to have a positive smear than culture-positive MTBC patients (6.1% versus 54.4%, aOR, 0.04, 95% CI: 0.02-0.10, *p <0.001*). Proportion of patients receiving Anti-retroviral therapy among NTM and MTBC group, respectively, was 67 (29.4%) and 39 (21.7%), unOR 1.50, 95% CI: 0.96-2.37, *p=0.08* [58].

Species identification and Xpert MTB/RIF result for NTM isolates

NTM species were identified in sputum samples from 180/228 (78.9%) patients, including 7 mixed NTM species. *M. intracellulare* was the most common species isolated 109 (47.8%) [58]. Other NTMs commonly associated with pulmonary disease included are shown in table 7. The common environmental contaminant *M. gordonae* was identified in 16 patients (7%) [58]. Forty-eight (21.1%) NTMs could not be speciated by the current Hain GenoType CM and AS LPA that we used for testing. After excluding NTM isolates that were non-speciated and *M. gordonae*, 154 (67.5%) of the NTM isolates were potential pathogens. (Table 7) [58].

Among the 408 isolates from culture-positive specimens tested retrospectively, Xpert MTB/RIF test results were available for 219/228 (96.1%) of NTM isolates and 177/180 (98.3%) of MTBC isolates [58]. For three patients with NTM species (1.4%) the Xpert MTB/RIF result indicated MTBC detected with rifampicin resistance. One of these three patients with NTM species from which MTBC detected had a mixed NTM (*M. avium and M. intracellulare*) [58]. The other two had mycobacterium species that we were not able to speciate further with the current testing methods we used. The specificity of Xpert MTB/RIF in this setting of PLHIV with NTM isolate was 216/219 (98.6%) (Table 7) [58]. The sensitivity of

Xpert MTB/RIF among PLHIV with culture and genotypically confirmed MTBC isolates was 176/177 (99.4%).

Table 7. NTM isolated among PLHIV presenting with TB symptoms in Botswana and corresponding Xpert MTB/RIF result

			Xpert MTB/RIF Results			
NTM Species	**Number**	**Frequency**	**MTBC detected**	**MTB not detected**	**Not available**	**RIF resistant**
M. intracellulare	109	47.8%	0	109	0	0
M. gordonae	16	7.0%	0	16	0	0
M. malmoense	9	3.9%	0	9	0	0
M. simiae	8	3.5%	0	8	0	0
M. scrofulaceum	6	2.6%	0	6	0	0
M. fortuitum	6	2.6%	0	6	0	0
M. asiaticum	6	2.6%	0	6	0	0
M. avium	5	2.2%	0	5	0	0
M. genavense	2	0.9%	0	2	0	0
M. lentiflavum	2	0.9%	0	2	0	0
M. abscessus	2	0.9%	0	2	0	0
M. kansasii	1	0.4%	0	1	0	0
M. phlei	1	0.4%	0	1	0	0
Mixed NTM*	7	3.1%	1	6	0	1
Others†	48	21.1%	2	37	9	2
Total	**228**	**100.00%**	3	216	9	3

**Mixed species: more than one NTM species identified per isolate*
†*NTMs* 'that we were not able to speciate further using the current testing methods we used'

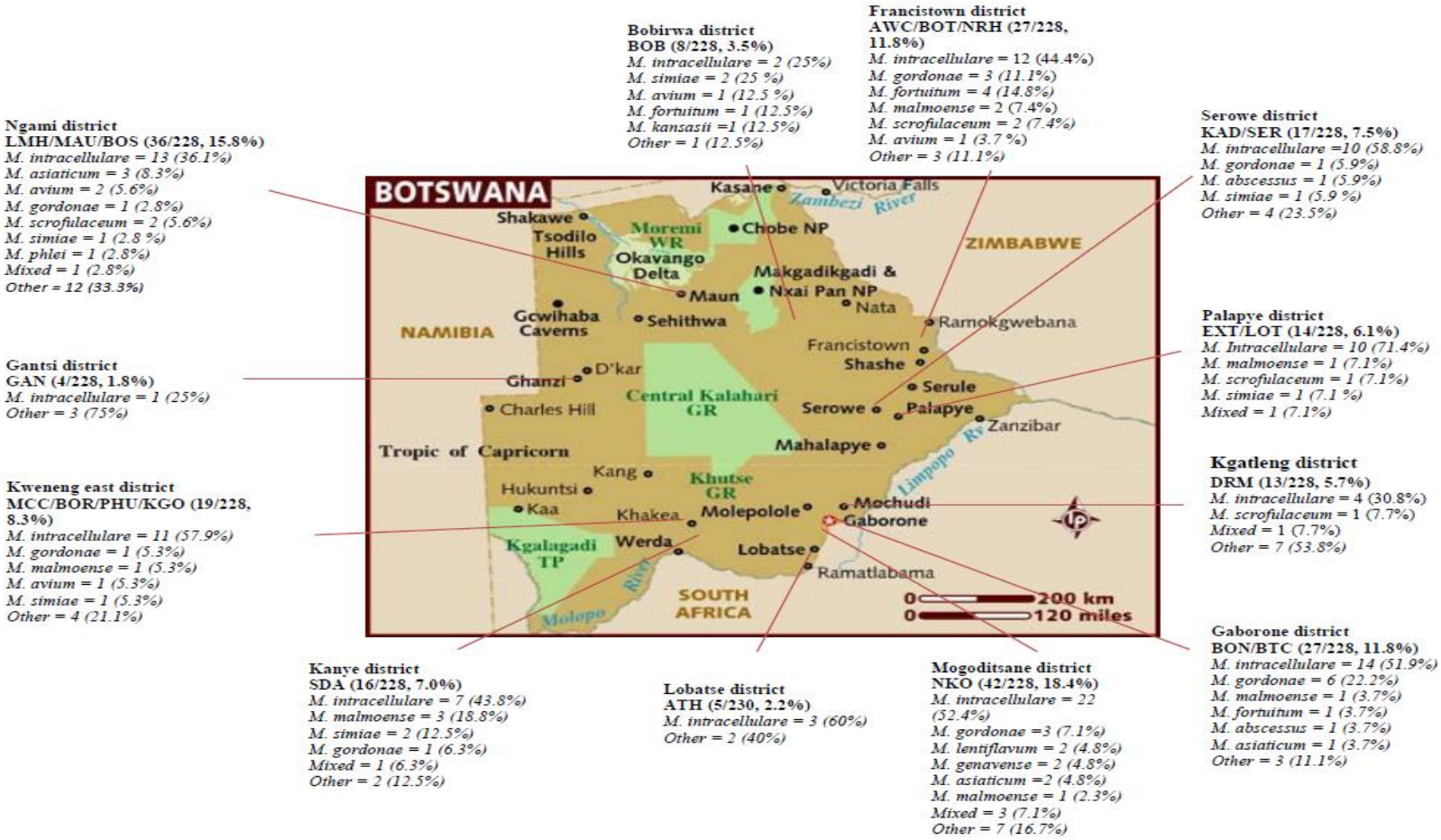
***Source: Agizew T**, et al. Higher-than-expected prevalence of non-tuberculous mycobacteria in HIV setting in Botswana: Implications for diagnostic algorithms using Xpert MTB/RIF assay. PLoS One 2017 22;12(12):e0189981.*

Geographic distribution of NTM by district

Fig. 6 displays the geographic distribution of NTM species. Twelve out of the 28 districts were included in this study [58]. *M. intracellulare* the most isolated NTM was identified in all the 12 districts, while *M. gordonae and M. malmoense*, the second and third most common were found in 7/12 (58.3%) and 6/12 (50%) of the districts, respectively [58]. *M. simiae* was found

in *4/12 (33.3%), M. avium and M. scrofulaceum* were in 3/12 (25%), *M. kansasii, M. phlei, M. genavense and M. lentiflavum* were in only one out of the 12 districts [58].

Fig. 6. Distribution of NTM among symptomatic PLHIV by district and clinical sites



Key to Fig. 6: Athlon Hospital = ATH, Area W Clinic = AWC, Bontleng Clinic = BON, Borakalalo Clinic = BOR, Boseja Clinic = BOS, Botswelelo Clinic = BOT, Broadhurst Traditional Clinic = BTC, Bobonong Primary Hospital = BOB, Deborah Retief Memorial Hospital = DRM, Ext 3 Clinic = EXT, Gantsi Hospital = GAN, Kadimo Clinic = KAD, Letsholathebe II Memorial Hospital = LMH, Lotsane Clinic = LOT, Maun Clinic = MAU, Molepolole Council Clinic = MCC, Kgosing Clinic = KGO, Nkoyaphiri Clinic = NKO, Nyangabgwe Referral Hospital = NRH, Phuthdikobo Clinic = PHU, SDA Hospital = SDA and Serowe Clinic = SER

***Source: Agizew T**, et al. Higher-than-expected prevalence of non-tuberculous mycobacteria in HIV setting in Botswana: Implications for diagnostic algorithms using Xpert MTB/RIF assay. PLoS One 2017 22;12(12):e0189981.*

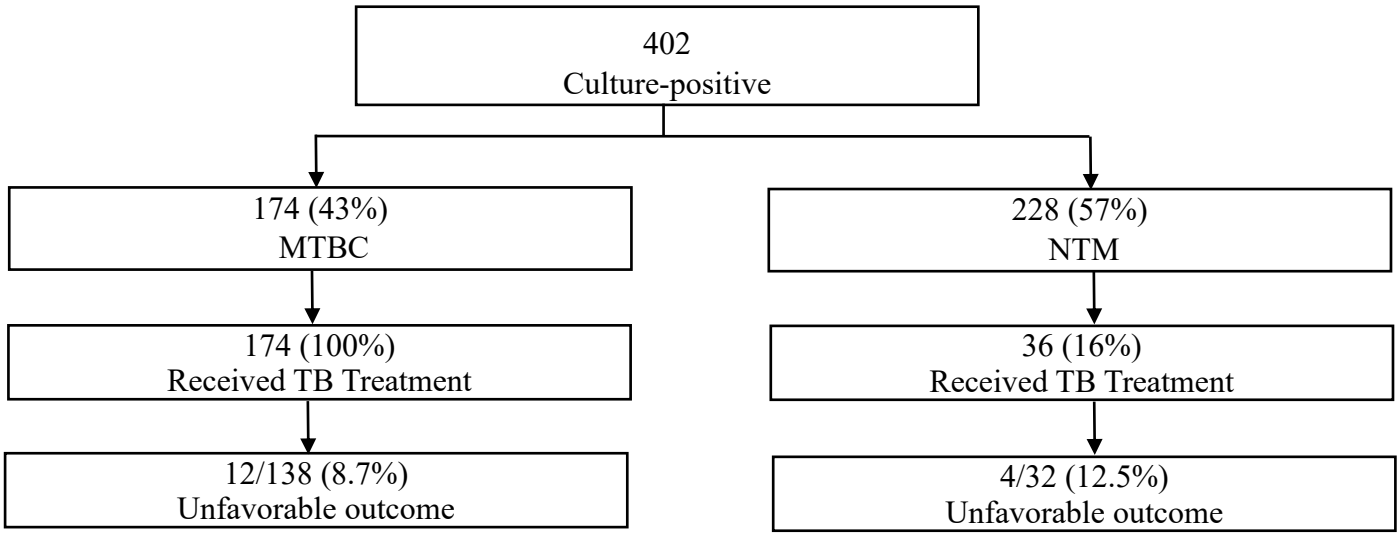
A total of 40 water samples were cultured from the water source used in the media preparation room and water from the deionizer that was used to prepare reagents. Ten percent (4/40) of results phenotypically indicated NTM positive-culture [58]. When further tested using Hain GenoType CM/AS line probe assays no NTM was isolated ruling out contamination from the source of water used for the preparation of all reagents and processing in the culture laboratory [58].

4.5. Treatment outcomes among people living with HIV with mycobacteria species, non-tuberculosis mycobacteria versus *Mycobacterium tuberculosis*, in Botswana (study four (a).

Summary result from study four, point (a) reports on the treatment outcomes of NTM treated with anti-TB treatment compared with patients with MTBC treated for drug sensitive TB is presented herewith. This was a unique opportunity to repost as some of the patients screened positive for TB symptoms, tested negative with Xpert MTB/RIF and positive NTM culture received anti-TB treatment at the discretion of the treating clinicians.

From August 2012 to November 2014, 1940 PLHIV with TB symptoms submitted ≥1 specimen [52]. Among these, 402 were culture-positive indicating 228 NTM and 174 MTBC (Fig. 7). Characteristics of patients with positive culture for MTBC and NTM treated with anti-tuberculosis treatment were not statistically significant (Table 8). Thirty-six (16%) of 228 patients with NTM and 174 with MTBC received TB treatment. TB treatment outcome was available for 32 NTM and 138 MTBC, unfavorable outcome (lost to follow-up, death, failure) was higher with 12.5% NTM versus 8.6% MTB, *p=0.51,* respectively, though the difference was not statistically significant (Fig. 7, Table 9). For 34 of the 36 treated NTM patient isolates were available and tested by Xpert MTB/RIF; all results indicated MTBC not detected. Among 228 NTM patients, those with body-mass index <18.5 (adjusted OR (aOR) 2.5, 95% Confidence Interval (CI), 1.31-4.76), weight-loss (aOR 1.72, 95% CI: 1.00-2.94) and TB history (aOR 3.86, 95% CI: 1.12-13.3) were more likely to be treated for MTBC.

Fig. 7. Unfavorable treatment outcomes among culture positive MTBC and NTM



***Source: Agizew T**, et al. Treatment outcomes among people living with HIV with non-tuberculosis mycobacteria versus mycobacterium tuberculosis in Botswana. Oral presentation 48th Union World Conference against TB and Lung Disease, Guadalajara, Mexico. Abstract No. OA-121-12. October 12, 2017.*

Table 8. Characteristics of patients with positive culture for MTBC and NTM treated with anti-tuberculosis treatment

	NTM			MTBC					
Characteristics	N	n	%	N	n	%	aOR	(95% CI)	*p value*
Age ≤ 35	36	15	41.70%	174	75	43.10%	1.26	0.78-2.03	0.32
Gender, female	36	15	41.70%	174	82	47.10%	0.78	0.37-1.63	0.48
CD4 count <200	36	17	47.20%	171	88	51.50%	1.70	0.68-4.29	0.23
BMI <18.5	36	22	61.10%	174	68	39.10%	2.61	0.85-8.02	0.09
HgB <10 mg/dl	29	5	17.20%	151	46	30.10%	0.35	0.07-1.75	0.18
TB Symptoms									
Cough	36	25	69.40%	174	140	80.10%	0.66	0.17-2.58	0.52
Fever	36	11	30.60%	174	85	48.90%	0.68	0.19-2.45	0.52
Night sweats	36	9	25.00%	174	81	46.60%	0.55	0.09-3.23	0.48
Weight loss	36	21	58.30%	174	118	67.80%	1.05	0.34-3.21	0.92
History of TB, Yes	35	10	28.60%	174	23	13.20%	3.50	0.77-16.0	0.10
Smear positive	36	7	19.40%	174	95	54.60%	0.13	0.04-0.46	0.004

***Source: Agizew T**, et al. Treatment outcomes among people living with HIV with non-tuberculosis mycobacteria versus mycobacterium tuberculosis in Botswana. Oral presentation 48th Union World Conference against TB and Lung Disease, Guadalajara, Mexico. Abstract No. OA-121-12. October 12, 2017.*

Table 9. TB treatment Outcome among PLHIV with Positive culture for MTBC and NTM treat with Anti TB Treatment

Treatment outcomes	NTM n=36	MTBC n=174	P value*
Unfavorable outcome	4 (12.5%)	12 (8.7%)	0.51
Favorable outcome	28 (87.5%)	126 (91.3%)	
Sub Total	32	138	
Transferred out or not evaluated	4	36	

*Adjusted for intra-site correlation

***Source: Agizew T**, et al. Treatment outcomes among people living with HIV with non-tuberculosis mycobacteria versus mycobacterium tuberculosis in Botswana. Oral presentation 48th Union World Conference against TB and Lung Disease, Guadalajara, Mexico. Abstract No. OA-121-12. October 12, 2017.*

4.6. Treatment-outcomes, diagnostic and therapeutic impact: Xpert MTB/RIF versus smear systematic review and meta-analysis (study five)

Summary result from study five is a systematic review and meta-analysis that is a synthesis of key impacts (treatment outcome, diagnostic or therapeutic) that were expected from the new diagnostic molecular technology that some even described as a 'revolutionary change' in TB world. The evidences, particularly towards improvement of TB treatment outcome, however, do not seem to show that expectations were met or assumption materialized. With that the systematic review and meta-analysis paper attempt to bring forth potential area of interventions that may be added to the body of knowledge of similar interest for those who would like to carry 'the torch' further and consider an intervention or a research. All results presented under 4.6 were per published paper [59].

A total of 683 unique citations were identified with 89 reporting on either outcome or impact (Fig. 8) [59]. Of these, 13 citations on drug-sensitive pulmonary were fully reviewed and a total of 43, 594 TB patients and 4825 with known TB treatment outcome were included for systematic review and meta-analysis, respectively [59].

Data for the citations used for systematic review and data for the four citations used for meta-analysis were from randomized clinical trials [25, 36, 38, 40, 43, 64-67] (Table 10). Compared between those diagnosed using Xpert MTB/RIF versus those diagnosed with smear: (1) patient

level treatment outcome among TB patents was reported in 46%, 6/13, of citations [36, 38, 60, 65, 67, 68] (Table 10), and in 31%, 4/13, citations among presumptive TB patients [10, 27, 28, 32], (2) the total number of patients diagnosed as TB was reported in 23%, 3/13, of citations [68-70] and proportion of TB patients diagnosed among presumptive TB in 46%, 6/13, of citations [25, 36, 38, 43, 66, 67]; (3) proportion of bacteriologically-confirmed TB was reported in 46%, 6/13, of citations among TB diagnosed [40, 64, 66, 68, 70, 71] and in 23%, 3/13, of citations among presumptive TB [25, 38, 69]; (4) proportion of TB patients empirically treated among TB patients was reported in 31%, 4/13, of citations [11, 27, 31, 33] and in 8%, 1/13, of citations among presumptive TB [36]; (5) proportion of loss-to-follow-up among presumptive TB was reported in 15%, 2/13, of citations [43, 67] and (6) time to diagnosis was reported in 23%, 3/13, [66-68] and time to treatment in 39%, 5/13, of citations [25, 38, 66-68].

Meta-analysis

The four citations that fulfilled the criteria for meta-analysis were from Botswana (one), Brazil (one) and South Africa (two). The data from these citations were summarized in Table 10 and 6.22 [38, 40, 64, 65]. Combined, a total of 4825 TB patients, 55% (2,675/4,825) diagnosed using Xpert MTB/RIF and 45% (2,150/4,825) by smear, were initiated on anti-TB treatment and had a known treatment outcome. The bivariate analysis showed no statistically significant difference on treatment outcomes between TB patients diagnosed using Xpert MTB/RIF and smear. Of the 4,825, 20.2% (541/2,675) and 21.9% (470/2,150) reported unfavorable outcomes among patients diagnosed using Xpert MTB/RIF and smear, respectively. The pooled analysis showed a RR of 0.92 (95% CI, 0.82-1.02) (Fig. 9). Statistical heterogeneity was low among the citations (I^2=0.0%, *p = 0.91).*

TB treatment outcomes

Out of the 13 reviewed citations, six reported on patient-level treatment outcomes among TB patients, four of which on complete treatment outcomes allowing classification as favorable or unfavorable; and treatment outcome was not different with statistical significance among those diagnosed using Xpert MTB/RIF versus those diagnosed with smear [59]. Yoon *et al* and Mupfumi *et al* reported on mortality at two and three months, respectively, among TB patients with no difference between smear and Xpert MTB/RIF group (Table 10) [38, 40, 64, 65, 67,

68]. Another four reported among presumptive TB that showed no mortality benefit at six month [36, 43, 66] or at three months [66]. Cox *et al* demonstrated superiority of Xpert MTB/RIF to smear (21.9%, 215/982 versus 17.5%, 176/1003, RR, 1.25, *p=0.032*) on treatment success (measured as favorable outcome) among presumptive TB patients [38].

Reviewed diagnostic and therapeutic impact: Xpert MTB/RIF versus smear

TB patients diagnosed

Of the three citations that reporting the total number of TB patients diagnosed [68-70], only Sachdeva *et al* reported 16% more TB patients diagnosed among Xpert MTB/RIF than smear (134/ 100, 000 versus 116/100, 000 population, RR 1.16, *P<0.001*). Though not statistically significant, Creswell *et al* reported an 8.5% lower than expected annual pulmonary TB notifications in the second year of introducing Xpert MTB/RIF introduction compared to baseline (pre-intervention; 4589 vs 5123 TB notifications) [70]. Of six studies done among presumptive TB cases, only Cox *et al* demonstrated higher proportion of TB diagnosed in Xpert MTB/RIF arm than routine (smear), (28%, 277/982, versus 23%, 299/1003, RR 1.44, *P=0.013*). The remaining five citation, no incremental benefit (i.e. increase TB case detection) observed, including Durovni *et al* where result presented per 100, 000 (79.6 versus91.7 per 100, 000, smear and Xpert MTB/RIF, respectively, *p=0.12*) rather than proportions [25, 36, 38, 43, 66, 67]. From Theron *et al*, compared to smear the effect of Xpert MTB/RIF on incremental value was demonstrated only within the first three days and the benefit no longer seen at day 14 (41%, 303/744 versus 39%, 292/758, *P=0.38*) and thereafter at day 28th and day 56th [36].

Empiric treatment

Five citations reported on empiric TB treatment [36, 61-64], in two out of four citations Xpert MTB/RIF reduced empiric treatment among TB patients [7%, 9/125 and 15%, 27/178 by Yoon *et al* and 17%, 4/24 and 56%, 9/16 by Calligaro *et al*] [62, 64]. In one citation Xpert MTB/RIF reduced empiric treatment among presumptive TB patients [17%, 130/744 and 26%, 197/758 by Theron *et al*] [36], compared to smear.

Bacteriologically-confirmed TB

In seven (78%) out of nine citations Xpert MTB/RIF was superior to smear in confirming TB bacteriologically [25, 36, 38, 43, 66, 68]. Cox *et al*, Durovni *et al* and Sachdeva *et al* reported bacteriologically-confirmed TB among presumptive TB and the rest six among TB patients. In Yoon *et al* and Calligaro *et al*, where bacteriological evidence was similar between Xpert MTB/RIF and smear, all TB patients included in these two analyses were hospitalized patients unlike the rest of the citations where Xpert MTB/RIF proved superior [59].

Time to diagnosis and time to treatment

Compared to smear, Xpert MTB/RIF significantly reduced delay in diagnosis in two of the three citation that measured time to diagnosis [66-68] and reduced time to treatment in one out of five citations [25, 38, 66-68]. Among the five citations Cox *et al* and Durovni *et al* from South Africa and Brazil, respectively, reported time to treatment without measuring time to diagnosis and Xpert MTB/RIF was useful on increasing number of TB patients who initiated treatment early in Brazilian setting, compared to smear [25, 38]. Theron *et al* reported the proportion of TB patients diagnosed and initiated on treatment but did not report the number of days [59]. Theron *et al* measured proportion of TB patients diagnosed and initiated TB treatment in that Xpert MTB/RIF increased number of TB diagnosed for those patients diagnosed and treated in the first days of sputum collection. On week two, four and eight, the proportion of TB patients diagnosed and initiated on treatment were not with statistically significant among patients diagnosed using Xpert MTB/RIF and those diagnosed using smear. Mortality at two and at six months was not statistically significant among patients diagnosed using Xpert MTB/RIF and those diagnosed using smear, irrespective of time to treatment initiation [36]. In none of the citations, improvement in diagnostic delay or early treatment initiation translated to an improvement in TB treatment outcome or mortality [59].

Loss-to-follow-up

Among the two citations reported on loss-to-follow-up among presumptive TB patients [43, 67] Xpert MTB/RIF showed no effect on reducing loss-to-follow-up. In Mupfumi *et al report* loss-to-follow-up was 15% (32/ 214) versus 18% (38/210), *p=0.38* and in Churchyard *et al* 0.99% (23/2,332) versus 1.08% (25/2324), p *value* NA [59].

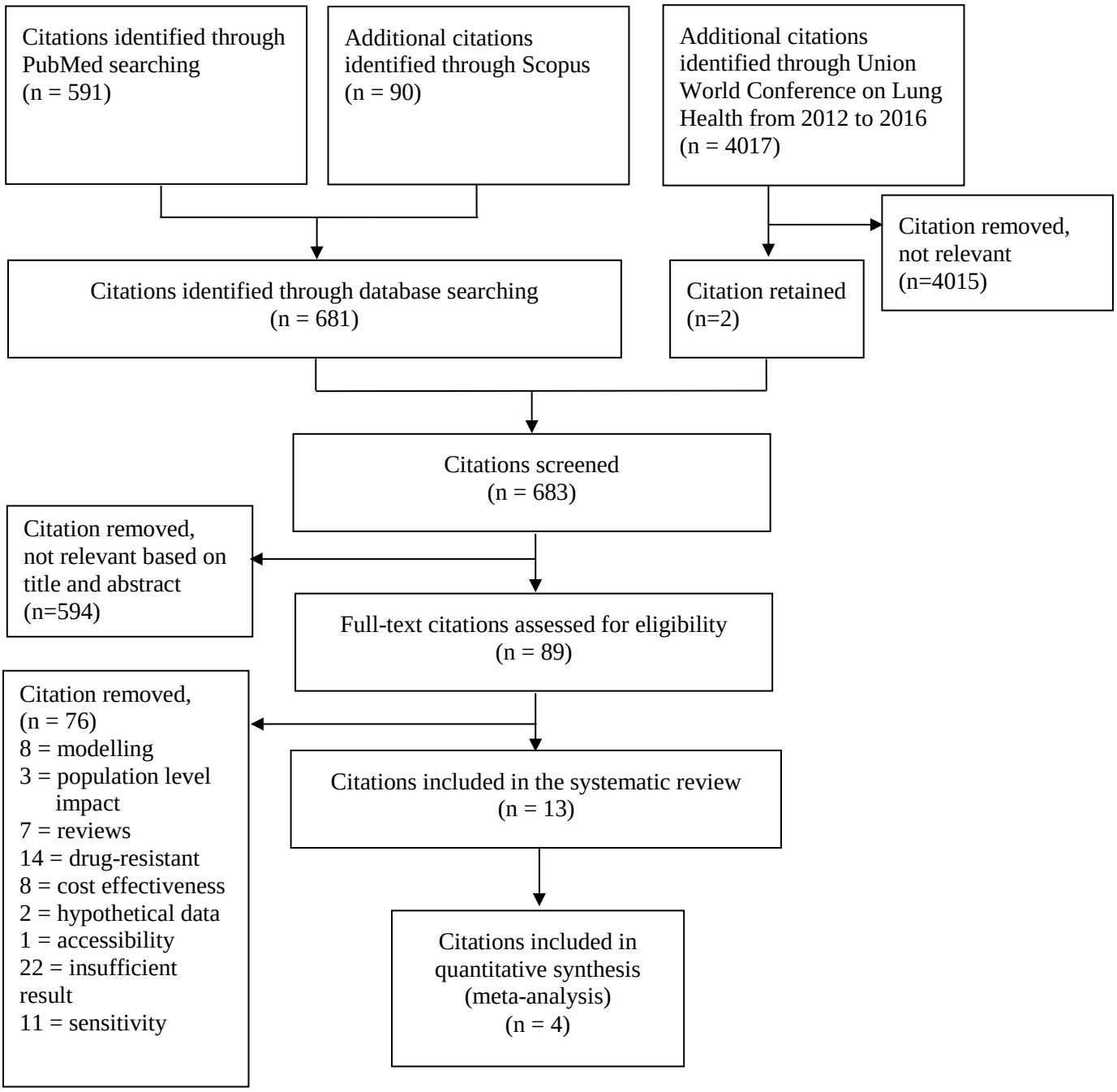
Table 10. Treatment outcome measured among tuberculosis patients

Author	Publication Year	Country	Design	Sample Size	Settings	Treatment outcome*	N, TB patients (smear)	n (%)	N, TB patients (Xpert MTB/RIF)	n (%)	RR	95% CI	p value
Yoon [68]	2012	Uganda	PC	477	In-patients	mortality at 2 months	153	26 (17.0%)	99	14 (14.0%)	-	-	0.80
Cox [38]	2014	South Africa	CRT	1,985	Out-patients, clinic	Unfavorable outcome§	204	28 (13.7%	249	31 (13.7%)	-	-	NA
Mupfumi [66]	2014	Zimbabwe	RCT	424	Out-patients, HIV clinic at hospital	Mortality at 3 months	172	17 (10.0%)	182	11 (6.0%)	-	-	0.19
Theron [36]	2014	South Africa, Zambia, Tanzania, Zimbabwe	RCT	1,502	Out-patients, tuberculosis clinic	Mortality at 2 months	324	26 (8.0%)	321	14 (4.0%)	-	-	0.054
Trajman [40]	2015	Brazil	CRT	4,088	In-patients and out-patients, clinic	Unfavorable outcome	1,676	409 (24.4%)	2,015	444 (22.0%)	-	-	NA
Agizew [63]	2015	Botswana	CRT	6,041	Out-patients, HIV clinic	Unfavorable outcome	39	8 (21.0%)	162	32 (20.0%)	1.03	0.63-1.71	
Fielding [64]	2015	South Africa	CRT	4,656	Out-patients, clinic	Unfavorable outcome	249	31 (12.5%)	213	25 (11.7%)	1.05	0.70-1.59	0.8

Note: The numerator and denominator of number of TB patients for smear and Xpert MTB/RIF group were taken as published in the citations.
Abbreviations: CRT=clustered randomized trial, RCT= randomized clinical trial, RR=risk ratio, CI=confidence interval, NA=not available
* Treatment outcome [favorable = cured, completed and unfavorable=failure, death (mortality), loss-to-follow-up])
§ Unfavorable = treatment failure, death, loss-to-follow-up

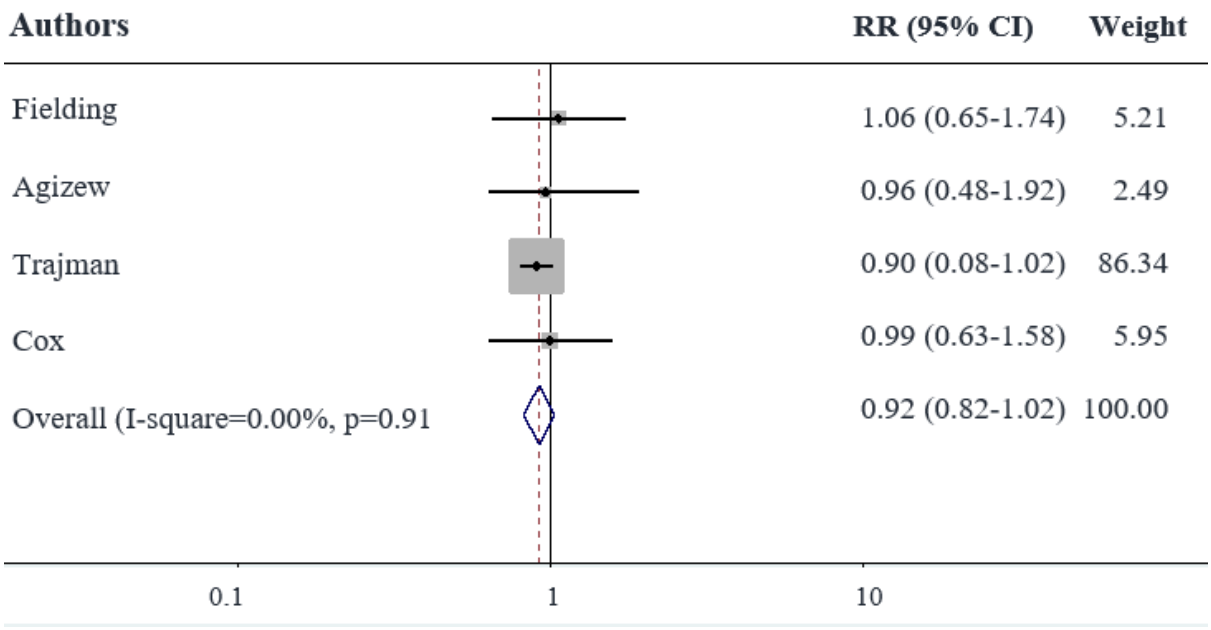
***Source: Agizew** T, et al. Treatment-outcomes, diagnostic and therapeutic impact: Xpert versus smear systematic review and meta-analysis. Intr Jr Tuberc Lung Dis 23(1):82-92. Jan 2019.*

Fig. 8. Flow chart of citations included for review and meta-analysis



***Source: Agizew** T, et al. Treatment-outcomes, diagnostic and therapeutic impact: Xpert versus smear systematic review and meta-analysis. Intr Jr Tuberc Lung Dis 23(1):82-92. Jan 2019.*

Fig. 9. Forest Plot of unfavorable treatment outcome among TB patients diagnosed by Xpert MTB/RIF versus smear



Abbreviations: RR=risk ratio, CI=confidence interval

***Source:* Agizew** *T, et al. Treatment-outcomes, diagnostic and therapeutic impact: Xpert versus smear systematic review and meta-analysis. Intr Jr Tuberc Lung Dis 23(1):82-92. Jan 2019.*

CHAPTER 5 DISCUSSION

Each paper has its own area to focus and thus the discussion presented by major points in each manuscript. Finally, the conclusion from all the papers summarized to consolidate the synthesis of the doctoral study.

5.1. Xpert MTB/RIF performance, higher incidence of an unsuccessful test at POC versus centralized laboratory, though the difference was not statistically significant: Higher failure rate with sample processing remains as major concern (study one).

The discussion part under 5.1 is presented as per the published paper from this PhD [52]. Before countrywide scale-up, we implemented Xpert MTB/RIF and assessed its performance under programmatic conditions in service of HIV care and treatment centres in Botswana [52]. Integrating Xpert MTB/RIF into routine systems required considerable investment in human resource training and infrastructure, and it was associated with challenges, several of which were difficult to anticipate [52]. Xpert MTB/RIF implementation in Botswana was feasible in both POC and laboratory settings [52]. Obstacles included failed UPS systems, rapid and regular rotation of personnel leading to the need for "in-house" training curricula, and delayed calibration of GeneXperts by local vendors [52]. Lessons learned during this pilot implementation of Xpert MTB/RIF continue to inform nation-wide scale-up with the expectation that implementation challenges will decline as the health system adjusts to the new diagnostic assay. However, continued monitoring is necessary to inform the long-term use of Xpert MTB/RIF in Botswana [52].

In this study, the cumulative incidence of unsuccessful Xpert MTB/RIF tests on sputum from HIV-infected presumptive TB patients was 15% [52]. Recent publications, involving multiple countries with high TB burden in Africa, Asia, and Europe, demonstrated overall Xpert MTB/RIF unsuccessful test incidence of 5-10% [23, 32, 36, 37]. Two of these studies reported Xpert MTB/RIF test results from laboratory settings. The other two studies included POC facilities in their report, one programmatic and the other in a controlled trial setting. Overall, unsuccessful test incidence in our study was one and half-fold higher than incidence

reported from programmatic settings and three-fold higher than incidence reported in a multicentre clinical trial from Southern Africa [24, 36].

Similar to Creswell *et al* and Ardizzoni *et al* [24, 37], about two-thirds of the unsuccessful tests in our study were recorded as error, followed by invalid (22%) and no result (9%) [52]. More than half of the errors were related to sample preparation processing or wrong sample volume added to the cartridge, suggesting that most of the unsuccessful tests were related to GeneXpert operators, rather than the instrument [52]. In the present study, there was a non-significant higher error rate when in-house trained operators were testing specimens possibly suggesting operator-related error might partly explain high incidence of unsuccessful test [52]. We are in agreement with Raizada *et al* that such errors could be minimized if trained Xpert operators follow SOPs correctly, handle sample preparation appropriately, and improve transfer of required minimum sample volumes into the Xpert MTB/RIF cartridge [32]. A regular monitoring visit from Xpert MTB/RIF trainers is critical to prevent potential errors [32, 52].

On the other hand, despite back-up generators and UPS, one in five errors linked to power supply suggesting that alternative sources of reliable power supply should be explored. The use of solar panels successfully piloted in India and Uganda and might work well in Botswana [32, 72]. One in seven errors was cartridge or module-related. Creswell *et al* reported 11–44% incidence of errors due to loss of signal or tube pressure (5011 code), and these results were from data collected before version G4 was widely available (March 2013) in the market [24]. Recent publications, however, reported that cartridge-related errors, particularly code 5011 due to signal loss error, considerably reduced following introduction of the G4 cartridge [20, 37]. Consistent with the recent studies, error incidence was 34% with G3 but 12% with G4 in our study [52]. This improvement is reassuring but further reductions in error rates are still needed. There were relatively few temperature-related errors (5%) in spite of summer temperatures regularly reaching $>35^0$C in Botswana. Sites were well-prepared in advance by equipping the GeneXpert locations with air conditioning; however improved monitoring and documentation of testing room temperature and GeneXpert maintenance (daily, weekly, and monthly) would be helpful to ensure optimal operating conditions [52].

There are only a few studies that compared POC and centralized laboratory-placed GeneXpert performance [52]. Theron *et al* in the Southern African trial reported a non-significant difference of unsuccessful test incidence at a POC versus centralized laboratory (5% versus 6%, *p=0.22*) [36]. Similarly, in the present study, the difference was not statistically significant between POC and centralized laboratory, though the rate was higher in the former. [52].

The 17% unsuccessful test incidence at POC in our setting, in contrast, was higher than that reported from Cambodia, where it was reported as 12%. The main difference was that in Cambodia, unlike in our study, laboratorians performed GeneXpert at the POC [24, 52].

Furthermore, in our study, power supply and temperature related issues (i.e., environmental factors) were lower at the centralized laboratory sites while sample-processing issues (i.e., operator-related factors) were higher at the centralized laboratory sites [52]. None of the differences was statistically significant. To clarify factors affecting GeneXpert performance at POC versus centralized laboratory, further studies focusing on potential determinants of GeneXpert performance are warranted [52].

Our result on Xpert MTB/RIF performance at POC versus at centralized laboratory has some limitations [52]. First, although we controlled for clustering by specifying random effect for study facilities, we did not control for other site- and operator-specific factors. For example, there may have been an association between unsuccessful test and certain GeneXpert operator. We were not able to assess the type and frequency of individual test results by GeneXpert operators because operators often shared the same password so there was no reliable identifying record available [52]. Second, information about interruptions of Xpert testing while processing, down time when the GeneXpert was not functioning, and reasons for interruption of testing and for how long interrupted were not systematically recorded. Third, room temperature at Xpert MTB/RIF testing sites was not consistently recorded [52]. Although a log sheet placed at each site to capture this information and GeneXpert operators taught how to fill out the form, information rarely recorded sufficiently to allow quantification of downtime and causes [52]. Fourth, six of our modules that had not passed calibration were replaced by refurbished modules [52]. South Africa has reported concerns about the performance of refurbished modules being associated with a higher incidence of unsuccessful tests [73]. However, we did not track which specimens tested by refurbished

modules and therefore are unable to evaluate if refurbished modules had higher incidence of unsuccessful tests than other modules [52]. Fifth, where qualified laboratorians or nurses were not available for Xpert MTB/RIF testing due to staff turnover in-house trained GeneXpert operators, without a need to formally pass the competency test, conducted some tests that might have negative effect on Xpert MTB/RIF test results [52].

5.2. At patient-level, among people living with HIV in Botswana, Xpert MTB/RIF reduced unfavorable treatment outcomes, compared to conventional smear, though not statistically significant (study two).

The discussion part under 5.2 is presented per the attached manuscript in Appendix 4. Endorsement of Xpert MTB/RIF as policy and its introduction into the TB diagnostics algorithm was relatively a smooth process in Botswana. The implementation of Xpert MTB/RIF together with the pragmatic clustered trial under routine programme condition, gave a unique opportunity to evaluate and compare Xpert MTB/RIF with smear. Xpert MTB/RIF demonstrated superiority on microbiologically-confirming TB, reducing time to treatment and reducing empiric treatment. At patient level, though testing using Xpert MTB/RIF resulted in more patients with favourable treatment outcomes compared to conventional smear, the difference did not reach statistical significance. In our settings, GeneXpert instrument or environmental related factors such as power supply and temperature that potentially affect Xpert MTB/RIF performance were controlled [52].

The reason for lacking such positive effect (reduced time to treatment initiation and reduced empiric treatment) translated into benefit of patient level treatment outcome is not clear. All patient in the present study were HIV infected, ART naïve at the time of enrolment with similar patient characteristics, including CD4 count, between the two arms suggesting that immunologic status or clinical presentations was not confounding the treatment outcome. Fever, that was close to two times higher among smear arm, was the only exception that neither affected the unfavorable outcome.

The present trial was one of the four randomized trials including from South Africa and Brazil discussing the TB treatment outcome benefits compared to smear [38, 40, 65]. Xpert MTB/RIF has been hailed for its high sensitivity and shorter turnaround time [25, 38].

However, in none of the four trials including the present study, Xpert MTB/RIF demonstrated improvement on TB treatment outcomes [38, 40, 65] measures as favorable and unfavorable suggesting that replacing smear by Xpert MTB/RIF is not sufficient enough to drive clinical outcome improvement. Furthermore, Theron *et al* and Yoon *et al* reported a two-month mortality [36, 68] and Cox *et al*, Calligaro *et al* and Churchyard *et al* reported a three and/or six-month mortality [38, 43, 67]. In all these recent publications, using Xpert MTB/RIF has not shown any mortality benefit compared to smear. The only report that shade light on mortality benefit was Auld *et al* where the study showed a 12-month mortality benefit that was observed over routine standard of care with enhanced care (defined as intensified case finding with additional staff support to actively tracing for patients who missed clinic appointments), rather than replacing smear with Xpert MTB/RIF. Auld *et al* suggested that intensified case finding and active tracing of TB patients to improve retention in care by healthcare workers was key factor for the mortality benefit [74].

Given available evidence, it is relatively clear that success of TB control will not be achieved by improvement in technology alone. TB diagnostics are a base for finding more TB cases and treating patients in a timely fashion. Stop TB Partnership and the WHO emphasizes that an evaluation of a new TB diagnostics is an essential component after WHO endorsement and introduction of a new diagnostics tool into national TB program activities. In an ideal world, the desired effect of new molecular diagnostics needs to be extended to an epidemiological impact such as reduced TB disease transmission at a population level at large that is beyond an effect at a patient level [75]. Over 145 countries implemented the Xpert MTB/RIF assay by 2016 [2]. With such progress in introducing Xpert MTB/RIF into national TB control activities failing to see patent level effect even in pragmatic trials particularly in HIV settings is worrisome implicating less likely success to a population level impact. Botswana has the second-highest HIV infection rate in the world, with one in five adults infected [4], and similar to other high HIV settings TB is a leading cause of mortality, as high as 40%, in this population [6, 9].

It is high time that the Botswana national TB program and similar settings consider another level of strategic innovation beyond reliance on technology. Auld *et al* report - intensified case finding and active tracing of TB patients to improve retention in care by healthcare workers - is an example of such. Despite a proven strategy TB preventive therapy (TPT) global uptake is slow. Only 13% of eligible children under five and 38% PLHIV newly

enrolled in care were receiving worldwide in 2016 [76]. Scale up of TPT is essential especially that a 37% mortality reduction was observed from the recent Badje *et al* report and the mortality benefit was independent of ART [77]. Per the new updated WHO recommendation establishing or scale-up of targeted TPT for even HIV negative households contacts of people with bacteriologically-confirmed TB in TB endemic countries is area to explore given the risk of TB among such group is higher than the general population [78]. Furthermore, with Xpert MTB/RIF potential to rapid diagnosis of drug-resistant TB, extrapulmonary TB diagnosis, pediatric TB and use of Xpert MTB/RIF Ultra assay with less infrastructure requirement considering further research to explore pre- and post-diagnostic test related factors in the TB diagnostic cascade in a health system is crucial [26]. In addition, a systematic review and meta-analysis summarizing evidences associated factors that are potential barriers to successful TB treatment outcomes of TB diagnosed by Xpert MTB/RIF versus smear is essential.

The result on Xpert MTB/RIF on patient level TB treatment in comparison with smear has some limitations. First, in both the Xpert MTB/RIF and smear groups, close to 20% of patients were either transferred out or not evaluated. Second, our analysis is a sub-study focusing on comparing treatment outcomes among patients with TB diagnosed via smear or Xpert MTB/RIF. Detailed results on culture and drug susceptibility tests were not included in this report because these findings will be published with the main XPRES study. Third, we were unable to control pre- and post-diagnostic test-related factors in the TB diagnostic cascade, such as delayed sputum sample submission, delayed sample transportation, lack of regular maintenance of GeneXpert instruments, and whether laboratories used the most recent version of the Xpert cartridge (G3 versus G4). Fourth, for some TB patients, diagnosis was during the follow-up period and the effect of follow-up visit on repeat TB screening, quality of sample collection and on the diagnosis of TB was not measured or adjusted in to the analysis. Lastly, we attempted to adjust for within facility difference when Wilcoxon rank-sum test analysis was conducted. However, STATA does not have a syntax for within facility adjustment when Wilcoxon rank-sum test analysis conducted. Thus, Wilcoxon rank-sum test analysis was not adjusted for clustering within clinics.

5.3. The yield of bacteriologically confirmed tuberculosis by Xpert MTB/RIF and smear: Does quality of sputum matter (study three)?

The discussion part under 5.3 is presented per the published paper from this PhD [57].
Our study demonstrated that among PLHIV with TB symptoms attending HIV care and treatment services, Xpert MTB/RIF was superior to smear at confirming the presence of MTBC in sputum as previously reported [38, 40, 43]. Sputum gross appearance and quantity, however, were not predictive of bacteriologically-positive sputum by Xpert MTB/RIF or smear suggesting that despite a minimum required volume of sputum – especially for Xpert MTB/RIF testing - suboptimal quality of sputum might have affect the yield of bacteriologically-confirmed TB [79, 80]. In our study, despite initial healthcare worker training the proportion of symptomatic patients that had sputum collected per national guidelines was suboptimal. Study staff reported that patients were not able to produce sputum and even when produced it had low volume (<0.2 mL) and quality particularly among those who were not coughing. These findings are consistent with a previous report by Ho *et al* [81] who analysed over 20,000 sputum samples collected as part of an active case finding project in Vietnam; they reported that the macroscopic quality was similarly not predictive of bacteriologically- positive sputum. In the present study, the only exception was sputum volume of 2ml to < 3ml that was predictive for a positive result when tested by Xpert MTB/RIF.

The percentage of samples classified as salivary in the present study was high at 43.3%, indicating potentially that, despite the training administered, a high proportion of patients were only able to produce salivary specimens. Recent studies from Kenya and Uganda also reported on the proportion of salivary sputa. In Kenya 44% of the samples were salivary and the study showed that salivary sputa had lower diagnostic yield than muco-purulent and mucoid sputa using Xpert MTB/RIF testing [82]. The study in Uganda examined presumptive TB patients screened with $\geq$ 2 weeks of cough; the proportion of salivary sputa was at 16% [83]. In both the Kenya and the Uganda studies, the proportion of salivary sample not affected by HIV status. In Uganda, Xpert MTB/RIF test conducted only among smear negative patients, and the diagnostic sensitivity, in contrast to our findings and those from Kenya, was significantly higher on salivary samples than mucoid sputa [83]. The Uganda findings on salivary sputum seem contrary to biological plausibility, showing higher

diagnostic yield with lower quality sputa. However, the report from Uganda was consistent after comparison of diagnostic accuracy in reference to mycobacterial culture (higher culture positive among salivary than non-salivary). Given the higher positivity among salivary sputum samples was confirmed by culture, we agree with Meyer *et al* that further study is essential exploring the possibility of potential dynamics affecting the Xpert MTB/RIF amplification in salivary sputum [83].

Ho *et al* emphasized that assessment of sputum quality is a neglected aspect of accurate TB diagnostics [84] Even though in the present study sputum quality was not predictive of diagnostic yield, we agree with Ho *et al* that TB programs should continue to train providers on high-quality sputum collection techniques. It is worth noting that despite the high cost of rolling-out, Xpert MTB/RIF implementation in the world is expanding [2]. If TB diagnostic algorithms do not encompass collection of quality sputum, such investments and their ultimate impact will potentially be compromised [82].

A wide variability in the proportion of patients with salivary sample (24-70%) among clinics suggests an inconsistency in sputum collection practices across the clinics. Bhat *et al* have shown an association of improved sputum quality and diagnostic yield [85]. It is time that TB screening and diagnostic algorithms include standardized methods of sputum collection and introduces a sputum collection system less prone to variability as suggested by Ho *et al* [84].

In some previous reports, improving sputum quality increased TB diagnostic yield [81, 84, 86-89]. Ho *et al* was able to collect 99% mucoid or muco-purulent sputa by using sputum quality colour scale [81, 84]; Alisjahbana *et al,* used instruction to patients, where patients were individually addressed on the importance of sputum examination and how to produce adequate samples; this technique demonstrated an improvement in sputum volume collection with thicker consistency and resulting in higher smear positivity rates [86]; Sicsu *et al* and Hirooka *et al* using similar methods as Alisjahbana *et al* showed higher quality and volume sputum samples that resulted in increased bacteriologically-confirmed TB diagnosis [87, 88]. Mhalu *et al* demonstrated that sputum submission instructional videos increased the yield of TB cases through better quality of sputum samples [88]. In addition, training of health care workers and laboratorians on standardized methods of sputum collection and assessment of adequate good quality sputum can improve sputum quality [85] and measuring a volume in millilitres using a graded reference container can facilitate appropriate volume collection

[85]. With these various methods, achieving improved quality and quantity of sputum were possible but well-designed studies are still needed to define a more comprehensive approach and standard.

While endeavouring to standardize sputum collection methods, under current clinical and laboratory practices, at least five reasons stand out about why salivary sputum should not be rejected: (1) a high percentage of salivary sputum are still being collected in clinical practice by health workers [82], (2) sputum specimen appearance and volume are poor 'negative predictors' of MTB in sputum [81, 90]; (3) a limited volume (only 1ml) of non-concentrated sputum maybe acceptable for Xpert MTB/RIF testing [91]; (4) recent study demonstrate higher sensitivity of Xpert MTB/RIF in salivary samples than mucoid [83] and (5) above all, to minimize any missed opportunities for TB diagnosis [92]. Sputum rejection criteria, that consider salivary sample as unsuitable for testing should be reconsidered, particularly when using Xpert MTB/RIF testing [84]. In the present study, if salivary samples had been rejected, 12% and 6% of TB cases identified by Xpert MTB/RIF and smear, respectively, would have been missed as bacteriologically-confirmed TB case.

Our report and discussion on the quality of sputum and a yield of bacteriologically-confirmed TB by Xpert MTB/RIF and smear has some limitations. Not all patients with TB symptoms were able to provide sputum, and for those who were able to provide sputum some did not receive sputum testing by both Xpert MTB/RIF and smear or were not tested at all. However, the patient characteristics among those tested by both Xpert MTB/RIF and smear and not tested by both or at all were not different with statistical significance. We also did not assess nor document laboratorians' skills in assessing the quality of sputa. Furthermore, there may have been inter and/or intra-operator variability in assessing sputum quality [93]. Lastly, some patients contributed more than one sputum sample during the follow-up period, and the effect of follow-up visit on repeated sample collection was not measured; and a potential lack of independence for each sample was not adjusted in to the analysis.

5.4. Higher-than-expected prevalence of non-tuberculous mycobacteria in Botswana settings: Implications for diagnostic algorithms using Xpert MTB/RIF assay (study four)

The discussion part under 5.4 is presented per the published paper from this PhD [58].
The results of this study demonstrated that among culture-positive sputum specimens collected from PLHIV with TB symptoms, 56% grew NTM [58]. To our knowledge, this prevalence of NTM among culture-positive specimens from PLHIV with TB symptoms is the highest described to date [47, 94-96], particularly in high HIV prevalence settings. Previous publications from African countries reported NTM prevalence among samples with a positive culture for mycobacteria ranged from 4-39% [27, 44, 47-49]. Unlike our study, the TB screening criteria used for most was ≥ 2 weeks of TB symptoms and the populations studied were mixed, including both HIV-infected and un-infected persons. The four-symptom TB screening used by Aliyu *et al* and the NTM prevalence was 16% among HIV infected and uninfected population of in-patient and outpatient [58].

In recent years, a few large population-based TB prevalence surveys both in Africa and Asia, have reported a high proportion of NTM (45-89%) among presumptive TB patients with sputum positive for mycobacteria by MGIT 960 [97, 98]. In these settings participants with presumptive TB were defined both by presence of symptoms and/or chest radiography with an abnormality in the lung or pleura [58]. These surveys also included both persons with and without HIV infection. Given the different population and screening criteria of the above studies, these results are not directly comparable to our study [58].

Sonnenberg, Conde and Lan *et als* reported 8%, 9.7% and 47% NTM prevalence among HIV infected patients with positive mycobacterial cultures, from South Africa, Brazil and China, respectively [47, 94, 95]. The sputum specimens in the China study that has closer prevalence with the present study, collected from patients with CD4 counts < 350 cells/mm^3, and had slightly different methods. The TB symptom screening methods used were before the current four symptom TB screen was recommended by WHO and the culture methods used was on a BacT/Alert 3D Microbial Detection System (bioMerieux, Craponne, France) [58]. In contrast to the report from Lan *et al* [94] our patients had higher median CD4 lymphocyte counts (205 versus 23 cells/mm^3) [58]. Given the higher risk of NTM with advanced HIV disease in Lan *et al* report and the higher median CD4 lymphocyte count in our study the prevalence of NTM is higher than expected [58]. Compared to the present study Sonnenberg *et al* and Conde *et al* reported lower NTM proportion from the HIV infected sub-group of studied population [58]. The Sonnenberg *et al* report was a case-control study amongst gold miner with a positive sputum mycobacterial culture tested using earlier version of liquid medium

(Broth culture systems such as the BACTEC 460 (Becton Dickinson Diagnostic Instruments, Sparks, Md.) [58]. While Conde *et al* reported from hospitalized patients, used solid culture medium (Lowenstein-Jensen) and the HIV testing was not offered to all patients rather to those with risk factors for AIDS with symptoms [58]. Moreover, similar to China report, both in Sonnenberg *et al* and Conde *et al* the TB symptom screening method used was not four symptom TB screen [58]. In the present analysis direct comparison with these previous studies was not done because of the various studied population, TB screening and culture methods used [58].

Liquid culture has been scaled up globally and increasingly used as the reference standard for confirming MTBC by referral laboratories. Use of liquid based culture methods are known to recover more NTM than solid based culture methods among persons with presumptive TB, including in high HIV prevalence settings [99] and this may contribute to the increased identification and reporting of NTM from large studies [58].

Botswana has the second highest HIV prevalence in the world: 19% in the general population [4]. Given the potential concern of pulmonary NTM infection and disease and unknown burden in this population, the findings have potential program implications [58]. In view of high HIV prevalence in the present study setting and rising NTM infection and disease in Africa, the findings are important to the larger global community [48, 49, 100]. HIV infection is a key risk factor [45, 94] that might explain of such high NTM prevalence in our study [58]. Other potential reason such as prevalent chronic diseases was not included in the analysis since it was beyond the scope of the present study [58]. Yet another area considered was contamination and an investigation of potential laboratory water sources of contamination at our referral laboratory indicated contamination during processing as less likely [101]. L. Buijtels *et al* from Zambia reported from a controlled study that the estimated rate of colonization in a mixed population of HIV infected and uninfected patients was 9% and the rate of disease was ≈2% [49].

While we acknowledge the potential clinical relevance of NTM colonization, infection and disease, since the present study not designed to address NTN diagnoses the America Thoracic Society criteria was not used and thus distinguishing NTM colonization versus disease was not possible [58]. However, in view of the epidemiology of NTM in Southern Africa, the high TB case notification rates (over 408/100,000 population) [102], and the high adult HIV

prevalence in Botswana [4], it is likely that our results point to NTM colonization or disease [58]. It is worth noting that among NTM group just above 6% of patients were smear positive for AFB and this remain to be a concern in settings without sputum culture facility and smear is the only diagnostic test [58]. Patients with smear positive for AFB and NTM positive culture are the likely to receive anti-TB treatment [58]. The present study demonstrated high specificity (98.6%) of Xpert MTB/RIF among NTM positive samples [58]. The concern of managing smear positive NTM patients with anti-TB treatment might be minimized if countries are shifting smear-based diagnostic algorithm to Xpert MTB/RIF-based diagnostic algorithm. To characterize the epidemiology of NTM and establish management strategy further study is essential in our settings [58].

In our study, the most common NTM species isolated was *M. intracellulare* followed by *M. gordonae* and *M. malmoense* [58]. Similar to reports from Hoza, Buijtels and Lan *et als.* [45, 49, 91], *M. intracellulare and M. gordonae* were among the top three species identified in culture-positive samples suggesting a similar predominance of these NTM species in different settings [58]. In contrast to Hoza, Buijtels and Lan *et als*, where no *M. malmoense was reported, we* found *M. malmoense* (4%) among the common NTMs. Aliyu *et al in* Nigeria also reported *M. malmoense* (3%) among culture-positive mycobacteria identified with NTM isolates [48].

Identifying *M. malmoense* among patients with NTM disease has clinical significance. Because of (1) this species may cause serious pulmonary morbidity reflecting a level of virulence unmatched by other NTM species [103], and (2) the two rpoB specific molecular beacons in *M. malmoense*, can give false positive Xpert MTB/RIF results with possible rifampicin resistance due to weak cross hybridization [8]. Rarely identified species of *M. lentiflavum* reported from Tanzania [45] and Zambia [49, 100] were also isolated in our setting. It is worth noting that *M. xenopi,* the third most frequent worldwide, was not identified in the present study and that was in agreement with Hoefsloot report which describes *M. Xenopi* as more common in Europe and Canada than in Southern Africa [103].

Studies from Sub-Saharan countries, including the present study, demonstrated that NTM species, *M. intracellulare, M. malmoense, M. abscessus and M. kansasi,* most commonly causing pulmonary infection are prevalent in Africa [45, 48, 49, 104]. Pulmonary NTM disease can have a similar presentation to pulmonary TB disease. The current TB diagnostic

algorithms in Botswana do not include screening, diagnosis and management of NTM [13]. Therefore, it remains difficult for clinicians to provide accurate diagnosis and treatment to patients with NTM disease [58]. Hoza *et al* noted that not addressing NTM diagnostic concerns would lead to potential under treatment of NTM [45], and as Maiga *et al* reported, possible over-diagnosis and over-treatment of drug resistant TB as well, especially in cases where NTM infection or disease co-exists with drug sensitive TB [50].

In our settings more than one percent isolates (3/219) of culture-positive NTM Xpert MTB/RIF test results indicated MTBC detected with rifampicin resistance [58]. Though the high specificity (98.6%) of Xpert MTB/RIF test to rule out TB among NTMs was reassuring, the three test results with MTBC detected and rifampicin resistance were concerning since it raises the possibility of multidrug-resistant TB diagnosis and treatment [58]. The three patients with NTM species that detected and rifampicin resistant MTBC based on Xpert MTB/RIF included a patient with mixed NTM (*M. avium and intracellulare)* and two patients with mycobacterium species [58]. The reason for the rifampicin resistance is unclear. For all the nine patients with *M. malmoense,* the species that has a potential for false Xpert MTB/RIF rifampicin resistance due to weak cross hybridization, MTBC was not detected by Xpert MTB/RIF test [14, 58].

There was no statistically significant difference of demographic characteristics of patients with NTM and MTBC in this study, except age [58]. Our finding is consistent with previous reports where age $\geq$50 was higher among patients with NTM than MTBC [48, 105-107]. Aliyu and Fusco-da-Costa *et al* reported that compared to patient with MTBC the average age among patients with NTM is higher [48, 105]. Prevots and Chung *et al* also described that generally the NTM prevalence increases with age compared to MTBC [106, 107], the likely reason being TB is a highly virulent pathogen, capable of infecting healthy individuals of all age and NTMs, in contrast, are opportunistic pathogens and patients need to have some risk factor for infection including age [105].

Clinically we observed that patients with MTBC positive culture were symptomatic in higher proportion than NTM [58]. In our study, fever, night sweats and weight loss were higher among patients with MTBC [58]. This is similar to reports from BaHammam *et al* (Riyadh, Saudi) and Kendall *et al* (Oregon (USA) [108, 109]. In the present study, however, the association became non-significant after adjusting for age, sex, CD4, BMI and history of TB

in a multi variable logistic regression model [58]. Findings from our study were consistent with a previous report that anemia was more common among TB patients than NTM [58, 110]. Available data on the association of anemia with NTM is limited, however there is possibility of disseminated NTM infection among patients with sickle cell anaemia [111].

Our result on higher-than-expected prevalence of non-tuberculous mycobacteria in Botswana settings study has some limitations [58]. First, the results analysed were from symptomatic patients who were able to submit sputum. Those patients who screened positive for TB symptom but who were not able to produce sputum were excluded and the prevalence of pulmonary NTM infection may be under reported [58]. Second, the two assays (Hain GenoType CM and AS) used were not able to further speciate the type of NTM for 21% of the isolates [58]. Third, the pulmonary specimens were collected from patients attending 22 HIV care and treatment clinics in 12 districts that do not necessarily represent the NTM prevalence in the whole country since the study facilities and patients included in this study were not randomly selected. However, these results do reflect a population of PLHIV enrolled in care and treatment that is typical in Botswana and findings provide useful epidemiological information about NTM infection that would be clinically relevant in the country [58]. Fourth, both smoking and mine current or history of exposure as predisposing factors predisposing factors for NTM disease (or colonization); and our data was not complete to report the proportions and NTM and MTBC [58]. Last, though we were able to assess and rule out the potential contamination from water sources at our TB referral laboratory we were not able to rule out the NTM colonization or environmental contamination from peripheral clinics where sputa were collected [58].

5.5. Does high prevalence of NTM affect tuberculosis treatment outcome (study four (a)?

In our study, we observed that patients with NTM presented with similar clinical presentation like patients with MTBC and treated with anti-tuberculosis treatment suggesting that where testing capacity for Xpert MTB/RIF, phenotypic culture and NTM speciation is limited misdiagnosis, delivery of sub-optimal treatment and outcome may occur.

A trend for non-significant higher unfavorable outcome observed among patients with NTM-positive culture than patients with MTB positive culture. The doctoral study was not designed to address such question. However, though difference of TB treatment outcomes were not statistically significant between patients with NTM and MTBC treated with anti-TB treatment, our findings highlight factors associated with an increased likelihood of receiving TB treatment among PLHIV with NTM. Patients with weight loss and previous history of TB were more likely to be treated with anti-TB despite Xpert MTB/RIF and culture showing no MTBC. Xpert MTB/RIF has the potential to rapidly rule-out NTM and avoid suboptimal treatment; further research is needed to evaluate such potential [112].

5.6. Why the Xpert MTB/RIF potential for diagnostic and therapeutic impact not translated to improvement in patient level treatment-outcomes (study five)?

The discussion part under 5.6 is presented per published paper from this PhD [59].
In an attempt to consolidate and address the overall aim of the doctoral study, we presented the synthesis of the point of interest – diagnostic and therapeutic impact of Xpert MTB/RIF versus smear in a systematic review and meta-analysis. To our knowledge, this is the first systematic review and meta-analysis of the impact of using Xpert MTB/RIF compared to smear, on patient level TB treatment outcomes (coded as favorable and unfavorable) in the diagnosis of drug sensitive TB [59]. The pooled results gathered from the four randomized trials included in the meta-analysis demonstrated no statistically significant difference on TB treatment outcomes among patients diagnosed with Xpert MTB/RIF compared to smear. This suggests that Xpert MTB/RIF has not been observed to significantly reduce unfavorable TB treatment outcomes [59]. There was no previous meta-analysis comparing drug-sensitive TB treatment outcomes between cohorts using Xpert MTB/RIF and smear [59]. Auld *et al* reviewed eight clinical trials was the only report available; Auld concluded that Xpert MTB/RIF showed no impact on the key patient level outcomes, mortality and morbidity [113]. Recent publications have demonstrated that Xpert MTB/RIF has a potential to increase the number of TB cases diagnosed [38, 69, 114], increase microbiologically-confirmed TB cases, and reduce time to diagnosis and treatment initiation [25, 38, 66, 68, 69, 115]. Beohme *et al* also demonstrated that shorter turnaround times resulted in substantially faster initiation of TB treatment, which resulted in a significant reduction in loss-to-follow-up [31]. Despite

such potential, why Xpert MTB/RIF did not result in a discernible impact in terms of improvement of clinical outcome remains unclear [59].

Theron *et al* hypothesized that potential benefits from Xpert MTB/RIF are masked by high levels of rapid empiric treatment [42], and many other reports agreed with such assumption [36, 38, 40]. In contrast, Churchyard *et al* reported that Xpert MTB/RIF actually has the potential to minimize the proportion of patients receiving empiric treatment [43]. Though clinicians cannot eliminate use of empiric treatment, Xpert MTB/RIF, compared to smear, has a higher sensitivity to accurately diagnose TB and a higher specificity to identify true negatives and this may be a benefit to patients who would be spared unnecessary TB treatment [43, 112, 115]. Creswell *et al* demonstrated an 8.5% reduction in empiric treatment in Nepal when Xpert MTB/RIF used in program settings [70]. Churchyard *et al*, Creswell *et al* and the present review did not support the assumption that clinicians continuing a high level of empiric treatment are masking the effects of Xpert MTB/RIF. Nonetheless, the concern 'why treatment outcome has not been affected by reduced empiric treatment' prevails [59]. The majority of reviewed citations in the present analysis indicated that use of Xpert MTB/RIF reduced empiric treatment, but no treatment outcome benefit observed [59]. On the other hand, it is worth noting that empiric treatment may have also some added value especially for TB in children and people with smear negative TB where the sensitivity of Xpert MTB/RIF is lower compared to sensitivity for smear positive or culture positive TB [59]. In such cases, it remains important to consider empiric treatment. The editorial from Lawn *et al*, in support of Theron *et al's* hypotheses, concluded that the effect and extent of empiric treatment on treatment outcomes warranted further evaluations in real-world settings [42, 115]. Currently there is an observational prospective study (ClinicalTrials.gov Identifier: NCT02729532) that began in 2016 in Zambia evaluating the effect of Xpert MTB/RIF on patient health outcomes and empiric TB Treatment among PLHIV. The study is expected to be completed in 2018, and the results will provide an opportunity *to* examine any association between use of Xpert MTB/RIF and empiric treatment [116].

In the present analysis, we reviewed diagnostic and therapeutic impact of Xpert MTB/RIF, and their effect on patient-level treatment outcome [59]. In the majority of citations (seven out of nine), though Xpert MTB/RIF increased bacteriologically-confirmed TB, the effect did not translate into increased numbers of TB cases treated or improvement in treatment outcome [25, 38, 64, 66, 68-70]. We are in agreement with Auld *et al* that a mere replacement

of smear with Xpert MTB/RIF is not enough to see the desired effect of highly sensitive new molecular testing [59, 113]. Another recent report from Auld *et al* seems to support this hypothesis; the study showed a significant 12 month mortality reduction when enhanced care (defined as intensified case finding with additional staff support actively tracing patients who missed clinic appointments) was added to routine standard of care, rather than only replacing smear with Xpert MTB/RIF. Auld *et al* suggested that the intensified case finding and active tracing of TB patients to improve retention in care by healthcare workers was a key factor for the mortality benefit [74].

The need for enhanced care discussed by Auld *et al* [113] is an issue of health system strengthening. Additional areas of concern, which was not included in our systematic review but has a potential to negatively affect Xpert MTB/RIF test results and thus treatment initiation, is quality of sputum [59]. Orina *et al* from Kenya and Meyer *et al* from Uganda reported concern with a higher than expected proportion of salivary sputum samples collected for Xpert MTB/RIF testing [82, 83]. Xpert MTB/RIF diagnostic test does not function in isolation; considering other health system and pre- and post-diagnostic test related factors in the TB diagnostic cascade is essential [26]. Three key intervention areas are important to address for successful Xpert MTB/RIF implementation: 1) The pre-diagnostic stage. Interventions may include training of Xpert MTB/RIF operators including proficiency tests, assessing potential diagnostic delay, both patient related (e.g. health care seeking) and health system (e.g. sample transport); assessing optimal placement of GeneXpert instruments to improve access to testing (high burden areas, point-of-care peripheral clinics or even at the community level if GeneXpert Omni is available) [59]. 2) The diagnostic stage. GeneXpert instruments should be regularly maintained and should use the latest version of Xpert cartridge (currently G4 or Ultra) [59]. 3) The post-diagnostic stage. Attention should be placed on turnaround time, early treatment initiation, reduced empiric treatment, and reduced loss-to-follow-up by actively tracking patients after treatment initiation [59].

While implementation of Xpert MTB/RIF as a highly sensitive and rapid diagnostic test remains appreciated in TB control efforts, patient related factors, quality sputum samples and stronger health systems seem to matter more than a need for an innovative technology alone [59]. The population level TB control success in high-income countries was achieved long before molecular testing was introduced and was not necessarily achieved because of the diagnostic technology used [117]. Having said this, the present study authors are not by any

means minimizing the need for the new molecular technology, Xpert MTB/RIF [59]. The Xpert MTB/RIF technology plays a very important role in the rapid diagnosis of drug-resistant TB, extrapulmonary TB diagnosis and childhood TB diagnosis [59]. Furthermore, the use of the Xpert MTB/RIF Ultra assay in settings with limited infrastructure is promising and should be further explored [59].

Our systematic review and meta-analysis have several limitations [59]. First, out of the 13 citations that reported on impact or treatment outcomes, only four fulfilled the inclusion criteria for the meta-analysis. All these citations were from randomized clinical trials but the covariate data on age, gender, HIV-status and previous history of TB were insufficient to include in the analysis [59]. Second, TB treatment outcomes for meta-analysis reported by Agizew and Fielding *et al* [64, 65] were adjusted for clustering, whereas data from the Cox and Trajiman trial reports were extracted and thus not adjusted for clustering [38, 40]. Third, the studies that measured delays in time to diagnosis or time to treatment used inconsistent definitions. Some studies measured from time of enrolment [38, 67], others measured from sputum sample collection [36] and some did not clearly define [25, 66]. Fourth, there were no data available on other potential factors that could influence patient care and outcomes. These data are like delay in sample transport, efficiency of patient referrals, proficiency of staff to conduct diagnostic tests, and adherence of clinicians to standard guidelines to screen, diagnose and treat patients, all of which could influence time to diagnosis and time to treatment [59]. Fifth, the population studied in the present analysis included a mix of HIV-infected and -uninfected persons, except for two citations which included only HIV-infected persons in the analysis [59]. Due to potentially compromised immune status, HIV infected individuals might possibly affect the treatment outcome [59]. However, the citations which included both did not report treatment outcomes by HIV status; thus, it is was not possible to see any impact difference between the HIV-infected and –uninfected groups [59]. Six, in the present analysis microbiological status of TB patients might potentially affect the treatment outcome; however, none of the citations included reported treatment outcome by microbiological status [59]. Lastly, though nine of the 13 citations reported in this analysis were from randomized clinical trials [25, 36, 40, 43, 64-67], the relative effect of study design potentially affected the impact of the interventions, as was highlighted in a review by Auld *et al* [113].

5.7. Conclusions

From this doctoral study, we learned that Xpert MTB/RIF was successfully introduced and its performance, as indicated by incidence of unsuccessful test, was not good at POC sites as in laboratory in the Botswana programmatic setting, though the higher unsuccessful test difference at POC has not reached to statistical significance. During Xpert MTB/RIF implementation challenges associated with introducing a new POC diagnostic test into the health system were observed, particularly nurses not always available to perform Xpert MTB/RIF testing at POC or showed occasional reluctance, as operating the GeneXpert was perceived as a non-nursing duty, until the task-sharing role was received well with further consultation [52]. Compared to other published reports high unsuccessful test incidence observed [52]. Repeating tests from leftover specimens has potential to provide valid test results, with correct adherence to SOPs, especially for sample processing errors [52]. Ensuring adequately trained staff retention and scheduling regular refresher trainings including competency assessments at testing sites will be important to minimize errors [52].

Our study presented GeneXpert performance; described error linked with codes to help elucidate causes, stratified the result by type of testing site, and examined trends over time [52]. Identified successes and challenges in our setting were important when Botswana MoHW was considering countrywide scale up of Xpert MTB/RIF [52]. Over 145 countries in the world have already invested in Xpert MTB/RIF implementation by December 2016 [2] and these findings contribute to initial reports of GeneXpert operation in resource-limited settings [52].

Moving in to the impact of Xpert MTB/RIF implementation in Botswana settings, the findings indicate that Xpert MTB/RIF reduced the overall unfavorable treatment outcomes among patients treated for drug-sensitive TB disease compared to smear, though the difference has not reached to statistical significance. Xpert MTB/RIF was useful for bacteriologically confirming TB, reducing time to treatment and empiric treatment as expected. However, despite such potential why Xpert MTB/RIF did not result in a significant impact in terms of improvement of clinical outcome remain unclear and warrants further research. One of the research areas not explored previously was whether ever-increasing NTM disease among PLHIV is affecting the treatment outcome when Xpert MTB/RIF is accessible to rule out NTM. This doctoral study though not power to address such important

question there was a trend observed towards negatively affecting treatment outcome. We had unique opportunity in the present study first to determine the prevalence of NTM among a positive sputum culture among PLHIV with TB symptoms than we found higher than ever reported at (56%) [58]. With this finding, first, the study suggests sputum culture and species identification for TB symptom-positive PLHIV with negative Xpert MTB/RIF results remains important to facilitate possible NTM diagnosis and hasten time to appropriate treatment [58]. Second, there was a possibility of mis-diagnosis of patients with NTM as MTBC and patients with NTM potentially sub-optimally treated with anti-tuberculosis treatment. From the present study, we learned that there was a non-significant higher unfavorable TB treatment outcome among patients with NTM culture positive than patients with MTB culture positive. However, whether such effect of NTM on treatment outcome contributed to failure to see impact of Xpert MTB/RIF over smear was not clear. Most importantly, though, our findings highlight factors associated with an increased likelihood of receiving TB treatment among PLHIV with NTM. Patients with weight loss and previous history of TB were more likely to be treated with anti-TB despite Xpert MTB/RIF and culture showing no MTBC. Xpert MTB/RIF has the potential to rapidly rule-out NTM and avoid suboptimal treatment; further research is needed to evaluate such potential.

Finally, from the doctoral analysis, also from systematic review/meta-analysis we conducted Xpert MTB/RIF implementation showed no statistically significant impact on clinical outcome compared to conventional smear [59]. Despite improved time to TB diagnosis, treatment initiation and/or empiric treatment patient-level outcomes were not significantly improved using Xpert MTB/RIF, compared to smear [59].

A recent report from Auld *et al* showed improvement patient level treatment outcome (mortality) over routine standard of care with enhanced care (defined as intensified case finding with additional staff support to actively tracing for patients who missed clinic appointments), rather than replacing smear with Xpert MTB/RIF [74]. Intensified case finding and active tracing of TB patients to improve retention in care by healthcare workers was key factor for the mortality benefit [40].

The need for enhanced care by healthcare workers discussed by Auld *et al* [37] is a health system strengthening issue. Also, an additional area of concern that has a potential to negatively affect Xpert MTB/RIF test result and thus treatment initiation was quality of

sputum as reflected in the present study and by Orina *et al* from Kenya and Meyer *et al* from Uganda. In these studies authors were concerned with a higher than expected proportion of salivary sputum samples collected for Xpert MTB/RIF testing [29, 41]. Xpert MTB/RIF diagnostic test would not function in isolation and considering other health system pre- and post-diagnostic test related factors in the TB diagnostic cascade is essential [42]. There are three key intervention areas. First, a pre-diagnostic stage such as training Xpert MTB/RIF operator with proficiency test passed, diagnostic delay either patient related by not submitting sputum early or delayed sample transportation; difficulty of access to testing that can be minimized by placement of GeneXpert instrument at a point-of-care peripheral clinic or even at the community level if GeneXpert Omni considered [59]. Second, a diagnostic test such as regularly maintained GeneXpert instrument that uses latest version of Xpert MTB/RIF cartridge (G4 or Ultra) [58]. Third, post-diagnostic stage such as turnaround time, early treatment initiation, reduced empiric treatment, reduced loss-to-follow-up by active patients tracking after treatment initiation [59].

While implementation of Xpert MTB/RIF as a highly sensitive and rapid diagnostic test remain appreciated in TB control effort patient related factors, quality sputum sample and health care workers within the health system seem to matter more than a need for an innovative technology [59]. The population level TB control success in high-income countries was achieved long before molecular testing introduced and was not necessarily achieved by use of a high diagnostic technology [43].

With the available evidence, efforts towards TB-control need more than just considering a replacement of smear with Xpert MTB/RIF [59]. Further implementation research is warranted to learn more about gaps in the existing health system [59]. Even though with Xpert MTB/RIF implementation, improvement in clinical outcomes has not yet been observed identifying areas of intervention for health system strengthening is a worthwhile exercise on an ongoing basis [59]. Within a wider context optimization of use of Xpert MTB/RIF to maximize benefits such as early diagnosis and management of drug-resistant TB, diagnosis of extrapulmonary TB and pediatric TB are also essential [59].

5.8. Recommendations:

- The result from this PhD demonstrated that Xpert MTB/RIF was effective when implemented at a POC compared to centralized laboratory, though there was a non-significant higher rate of unsuccessful tests, mostly related with sample preparation. We recommend that, in the Botswana settings, when scaling up Xpert MTB/RIF at POC standardized sample preparation procedures need to be emphasized.
- When scale up of Xpert MTB/RIF considered we recommend having planning for adequately trained staff is retained and regular refresher trainings including competency assessments at testing sites.
- The findings from the present analysis does not provide evidence that relying on technology, such as Xpert MTB/RIF, alone might lead to a promising direction in our fight with TB control effort. Further research on health system strengthening to improve TB diagnosis, to reduce empiric treatment and improve treatment among PLHIV is recommended understand potential contribution to mortality reduction from undiagnosed TB or TB diagnosed late.
- NTM prevalence was higher-than expected in the national TB reference laboratory culture reports among samples from PLHIV and thus further prospective study is recommended in Botswana to understand the epidemiology and prevalence of NTM disease in Botswana, especially among PLHIV.
- The current TB diagnostic algorithm does not consider NTM diagnosis and thus we recommend that the MoHW Botswana include NTM in the TB diagnostic algorithm, especially among patients with TB symptom and Xpert MTB/RIF or culture negative for MTBC.

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APPENDICES

Appendix 1: The list of variables for which data collected from GeneXpert (objective 1) and DISA (objective 4).

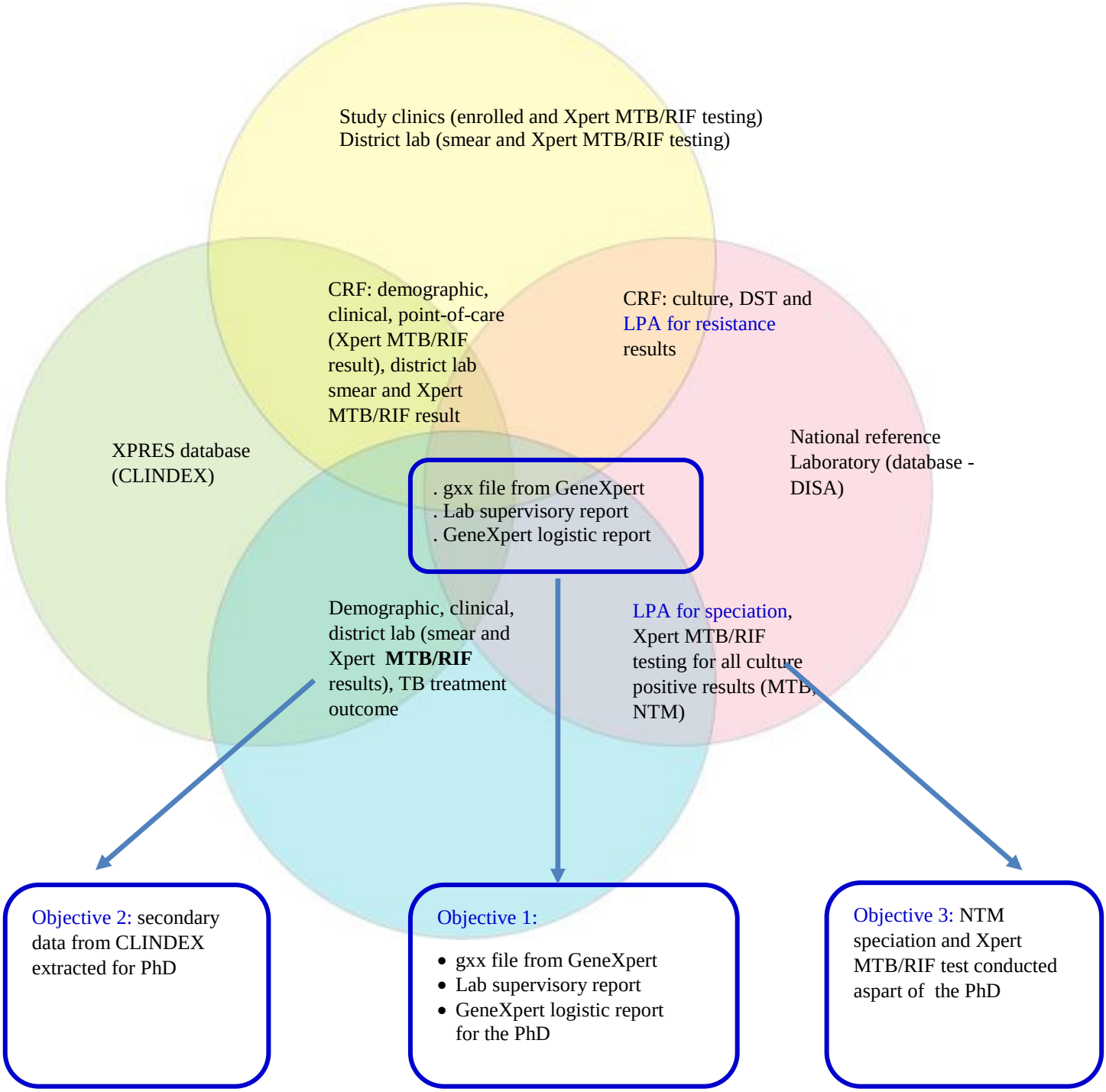
Data capturing tool and variables for objective # 1

Patient identification number e.g. 1000, 2000	Gene Xpert site	Date Xpert test performed	Resuls: Sucessful: MTB detected (Y/N)	MTB not detected (Y/N)	Rifampicin sensitive (Y/N)	Rifampicine resistant (Y/N)	unsuccessful: error (Y/N)	invalide (Y/N)	no-result (Y/N)	Indeterme diate (Y/N)	GeneXpert recorded error codes recorded (e.g.1001, 2032, 2034...)
1000	AWC	15-Jan-13	Y	N	Y	N	N	N	N	N	.-
1001	NKO	20-Jan-14	N	N	N	N	Y	N	N	N	1001

Data capturing tool and variables for objective # 3

Patient identification number e.g. 1000, 2000	Study site	MIGT - liquid culture positive result (Y)	MTB (Y/N)	NTM (Y/N)	Line Probe Assay result: Isoniazid sensitive (Y/N)	Isoniazid resistant (Y/N)	Rifampicin sensitive (Y/N)	Rifampicine resistant (Y/N)	NTM Speciated (Y/N)	NTM Species E.g. MAC	Xpert test result (sample from the positive culture): Xpert tested (Y/N)	MTB detected (Y/N)	MTB not detected (Y/N)	Rifampicin sensitive (Y/N)	Rifampicine resistant (Y/N)	Indetermdiate (Y/N)
1000	AWC	Y	N	Y	Y	N	Y	N	N	MAC	Y	Y	N	Y	N	N
1001	NKO	Y	Y	N	Y	N	N	N	N	MAC	N	N	N	N	N	N

Appendix 2: Venn diagram on data source



Appendix 3: Initial Xpert MTB/RIF implementation sites in Botswana

	District	Health facilities	Facility code	Level of health facility	Placement of Xpert MTB/RIF (POC or lab)	Xpert MTB/RIF operator
1	Ngami	Lethsolathebe memorial hospital	**LMH***	Referral hospital	Referral hospital lab	Lab tech
		Boseja clinic	BOS	Primary health care		
		Maun clinic	MAU	Primary health care		
2	Gaborone	Bontleng clinic	**BON***	Primary health care	District clinic lab	Lab tech
		Broadhurst traditional clinic	BTC	Primary health care		
3	Francistown	Nyangabgwe referral hospital	**NRH***	Referral Hospital	Referral hospital lab	Lab tech
		Arear W clinic	**AWC***	Primary health care	District clinic lab	Lab tech
	Francistown	Botswelelo clinic	BOT	Primary health care		
4	Kweneng East	Kgosing	KGO	Primary health care		
		Molepolole council clinic	**MCC***	Primary health care	POC	Nurse
		Borakalalo clinic	BOR	Primary health care		
		Phuthadikobo clinic	PHU	Primary health care		
5	Kweneng East - Mogoditsane	Nkoyaphiri clinic	**NKO***	Primary health care	POC	Nurse
6	Palapye	Exetnsion 3 clinic	**EXT***	Primary health care	POC	Nurse
		Lotsane clinic	LOT	Primary health care		

7	Serowe	Kadimo clinic	**KAD***	Primary health care	POC	Nurse
		Serowe clinic	SER	Primary health care		
8	Bobirwa	Bobonong primary hospital	**BOB***	Primary hospital	Primary hospital lab	Lab tech
9	Kanye	Seventh day adventist hospital	**SDA***	Primary hospital	Primary hospital lab	Lab tech
10	Gantsi	Gantsi primary hospital	**GAN***	Primary hospital	Primary hospital lab	Lab tech
11	Kgatleng	Deborah retief memorial hospital	**DRM***	Primary hospital	Primary hospital lab	Lab tech
12	Lobatse	Athlone primary hospital	**ATH***	Primary hospital	Primary hospital lab	Lab tech

* Health facility where Xpert MTB/RIF installed

Appendix 4: Scientific articles included in the integrated narrative