University of Kwazulu-Natal

Title: The impact of the introduction of direct first and second-line reflex testing in the management of drug-resistant Tuberculosis at Greytown Hospital, Umzinyathi district, KwaZulu-Natal

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2019

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By

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Submitted in fulfilment of the requirements for the degree of ***Master of Medical Science (Medical Microbiology)*** *in the School of Laboratory Medicine and Medical Sciences, University of Kwazulu-Natal*

In the College of Health Sciences

School of Laboratory Medicine and Medical Science

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Submission Date: 27 January 2020

Preface

This study was undertaken to assess the value and effectiveness of the changes in tuberculosis diagnostics in South Africa. The research is of importance in the field of medical microbiology and public health and aimed to provide previously unknown data that will aid stakeholders in decision-making regarding the diagnosis and management of drug resistant tuberculosis in a rural setting in South Africa.

Declaration

I ..... declare that

(i) The research reported in this dissertation/thesis, except where otherwise indicated, is my original research.

(ii) This dissertation/thesis has not been submitted for any degree or examination at any other university.

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Acknowledgements

Thank you to my Supervisors, Prof. Koleka Mlisana and Dr Marian Loveday who helped and supported me and kept me on the right track throughout this process. A thank you also goes to Sister Lee-Megan Larkan from the Greytown TB Facility who always made time to assist me when required. Thanks to the Greytown TB Hospital and the NHLS who made it possible for me to conduct this research. Also mention must be made to Ebenezer Ogunsakin who assisted me with the statistics. Lastly, thank you to the University of Kwazulu-Natal who funded this research.

Abstract

Background

Drug resistant tuberculosis (DR-TB) is a serious public health issue both globally and nationally, with South Africa and Kwazulu-Natal, in particular, being among the regions with the highest burden of DR-TB. Detecting drug resistance and initiating patients onto the appropriate therapy, in the shortest possible time, is of utmost importance to the effective management of DR-TB. The development of molecular diagnostic techniques allows for more rapid diagnosis of TB, as well as drug resistance, leading to earlier diagnosis and subsequent initiation onto appropriate treatment.

For phenotypic drug susceptibility testing (DST), the laboratory turnaround time is 4 – 6 weeks, thus patients are either initiated onto empiric and sometimes inappropriate treatment or have to wait to be initiated onto appropriate therapy, remaining untreated and infectious for extended periods of time. The introduction of GeneXpert testing revolutionised TB diagnostics as it allowed for diagnosis of TB whilst also providing susceptibility results for rifampicin within a few hours. Direct 1st and 2nd line LPA testing was included in the DR-TB management algorithm to further reduce the time to treatment initiation of multidrug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB). This also ostensibly reduces the amount of time a patient is transmissible for and improves treatment outcomes. This study was undertaken to assess the impact of the introduction of the direct 1st and 2nd LPA reflex testing on the management of DR-TB in the Umzinyathi District of Kwazulu-Natal.

Methods

The cohorts before and after the roll-out of direct 1st and 2nd line LPA testing were analysed for patient characteristics, diagnostic information, time to appropriate treatment initiation and treatment outcomes. Furthermore, the diagnostic tests were compared to ascertain if 1st and 2nd line LPA is comparable to phenotypic DST for drug susceptibility testing.

Results

There were 141 patients included in the 2015/2016 cohort before direct 1st and 2nd LPA was included in the algorithm, and 102 patients in the 2017/2018 cohort after its implementation. There was a significant decrease between cohort 1 and cohort 2, in the laboratory turnaround time for both 1st line LPA, which decreased from 36 days (IQR 23 – 60) to 17 days (IQR 11 – 30), respectively, and 2nd line LPA, which compared to phenotypic DST, decreased from 45 (IQR 23 – 67) to 21 days (IQR 12 – 50). Time to appropriate treatment initiation was similar across both cohorts for RR- and MDR-TB, from 8 days (IQR 5 – 13) to 9 days (IQR 7 – 29) in the second for RR-TB, and from 8 days in cohort 1 (IQR 6 – 20) to 12 days (IQR 6 – 50) in cohort 2 for MTB-TB. The time to appropriate treatment was significantly reduced in XDR-TB patients from 267 (IQR 145 – 796) to 62 days (IQR 45 – 182)

(*p=0.018*). Moreover, the treatment outcomes in XDR-TB improved after the roll-out of direct 1st and 2nd line LPA. Xpert, 1st line and 2nd line LPA performed well compared to phenotypic DST for antibiotic resistance detection.

Conclusion

The laboratory turnaround time and time to appropriate treatment initiation improved after the implementation of direct 1st and 2nd line LPA. Despite a delay in initiating therapy after laboratory diagnosis, there were positive impacts found regarding treatment outcomes of XDR-TB. Patients were initiated on the appropriate treatment, in response to 2nd line LPA results, in the first instance, which improved treatment success rates in XDR-TB patients.

Keywords: Mycobacterium tuberculosis, line probe assay, MDR-TB, XDR-TB, phenotypic DST

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ABBREVIATIONS

AFB acid fast bacilli

AFRO African regional office of the World Health Organization

ART Anti-retroviral therapy

CI confidence interval

DOTS directly observed therapy, short course

DR drug-resistant

DR-TB drug-resistant tuberculosis

DST drug susceptibility testing

DTD demonstration and training districts

INH isoniazid

LPA line probe assay

MDR-TB multidrug-resistant tuberculosis

MTDP Medium-Term Development Plan

NDoH National Department of Health

NHLS National Health Laboratory Services

NSP National Strategic Plan

NTP National Tuberculosis Programme

PCR polymerase chain reaction

RIF rifampicin

RR-TB rifampicin-resistant tuberculosis

SDG Sustainable Development Goals

TB tuberculosis

WHO World Health Organization

XDR-TB extensively drug-resistant tuberculosis

DEFINITIONS

Mono-resistant-TB	Resistance to one first-line anti-TB drug only.
RR-TB	Resistance to rifampicin, detected using phenotypic or genotypic methods, with or without resistance to any other anti-TB drugs.
DR-TB	This refers to disease which is resistant to one or more anti-tuberculosis drugs. Resistance is determined through laboratory confirmation of *in vitro* resistance to one or more anti-tuberculosis drugs. Drug-resistant TB develops when micro-organisms are not killed or inhibited by a specific antibiotic due to the selection of naturally occurring resistant mutants through inadequate therapy (too few medications, insufficient dosing and/or inadequate duration of therapy).
MDR-TB	A patient with multidrug-resistant TB has a strain of bacteriologically proven TB in which the bacilli show *in vitro* resistance to rifampicin and isoniazid, with or without resistance to other first line anti-TB drugs.
Pre-XDR-TB	A patient with bacteriologically proven TB in which the bacilli show *in vitro* resistance to rifampicin, isoniazid and either an injectable or a fluoroquinolone.
XDR-TB	A patient with extensively drug-resistant TB has a strain of bacteriologically proven TB in which the bacilli show *in vitro* multidrug-resistant TB together with resistance to any fluoroquinolones, plus resistance to one or more the following injectable anti-TB drugs: kanamycin, amikacin, capreomycin.
Pulmonary TB	Any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or the tracheobronchial tree.
Extrapulmonary TB	Any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs.
New patient	Has never been treated for TB or have taken anti-TB drugs for less than 1 month.
Recurrent	Has previously been treated for TB and were declared *cured* or *treatment completed* at the end of their most

	recent course of treatment and are now diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by reinfection).
Treatment after failure	Have previously been treated for TB and whose *treatment failed* at the end of their most recent course of treatment.
Treatment after loss to follow-up	Have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment.
PT1	Previously treated with 1st line anti-TB drugs.
PT2	Previously treated with 2nd line anti-TB drugs.
DR-TB Cured	A patient who has had a DR-TB culture conversion, received treatment for a total duration of >9 months, has had at least 3 consecutive negative TB cultures during continuation phase (at least 30 days apart) and there is no evidence of clinical deterioration
DR-TB Treatment completed	A patient who has had DR-TB culture conversion, received treatment for >9 months, has less than 3 consecutive negative TB cultures during continuation phase (at least 30 days apart) and there is no evidence of clinical deterioration
Loss to follow up	A patient whose treatment was interrupted for two or more consecutive months for any reason without medical approval
DR-TB Treatment failure	A DR-TB infected patient failed to undergo culture conversion by month 4 or two or more of the five cultures recorded in the final 6 months of therapy are positive, and the patient's clinical condition is deteriorating, or if a clinical decision has been made to terminate treatment early or to change the treatment regimen by adding more than two medicines due to poor clinical and/or radiological response or adverse events.

Died	A patient who dies from any cause during the course of DR-TB treatment
Transferred out	A patient who has been referred from the facility to another facility in another district, province or country, for whom the treatment outcome is not known
Moved out	A patient who has been referred from the facility to another facility in the same district, province or country, for whom the treatment outcome is not known.
Not specified	A patient recorded in the DR-TB register who does not have the necessary recorded data to enable classification of outcome any other category. They may be continuing extended treatment due to non-adherence or complications.
Successful outcome	Includes: Cured and Treatment completed
Unsuccessful outcome	Includes: Died, Treatment Failed and Loss to follow up,

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CHAPTER 1

1. Background and Literature Review

1.1. Defining the Clinical Problem: The Epidemiology of Tuberculosis

Globally, there were 10 million new cases of tuberculosis (TB) reported in 2018 (1). Of those, 500 000 were diagnosed with rifampicin resistant-TB (RR-TB). A further 78% of the RR-TB were multi-drug resistant-TB (MDR-TB). In the same period, there were an estimated 1.2 million TB related deaths (1). South Africa falls among the 30 high TB burdened countries that account for 87% of all TB cases globally (1). South Africa has the third highest number of reported TB cases and the fifth highest number of undiagnosed cases, and amongst the highest reported incidence and prevalence of both TB and MDR-TB in the world (1). Within South Africa, KwaZulu-Natal has the highest incidence of both MDR-TB and extensively-drug resistant TB (XDR-TB) cases (2).

In 2018, South Africa had 227 999 TB cases, 3.4% of which were new MDR-TB cases (1). According to World Health Organization (WHO) statistics, South Africa has a 77% treatment success rate for TB, and the incidence of TB decreased by 5% from 2017 to 2018 (1, 3). However, treatment success rates decrease with increasing resistance, e.g., MDR-TB and XDR-TB success rates are 54% and 58%, respectively, below South Africa's TB eradication goals (3) (Table 1). In 2018, there were 13 199 MDR/Rifampicin resistant-TB (RR-TB) and 553 XDR-TB cases. Of these, 9 558 (72%) MDR/RR-TB patients were started on treatment compared to 539 (97%) XDR-TB patients (3). In the National Strategic Plan on HIV, STIs and TB: 2017-2022 (NSP), targets enabling South Africa to achieve the Sustainable Development Goals are set. If the country is not able to increase the number of patients diagnosed with MDR- and XDR-TB and improve the current success rates for all TB, these targets will not be met. (1, 4).

		2016	2017	2018
Total TB cases		244 053	227 224	227 999
DR-TB care	MDR/RR-TB cases	19 073 (7.8%)	15 986 (7.0%)	13 199 (5.8%)
	MDR/RR-TB cases started on treatment	11 192 (59.7%)	10259 (64.2%)	9 558 (72.9%)
	XDR-TB cases	967 (0.4%)	747 (0.3%)	553 (0.2%)
	XDR-TB cases started on treatment	628 (64.9%)	463 (62.0%)	539 (97.5%)
Treatment Success	New & recurrent cases	81%	82%	77%
	MDR/RR-TB	54%	55%	54%

		2016	2017	2018
	XDR-TB	27%	48%	58%

Table 1: Drug resistant tuberculosis incidence and successful treatment outcome rates for South Africa in 2016, 2017 and 2018 (1, 3, 5).

(Definition of Abbreviations: TB=Tuberculosis, DR-TB=drug resistant tuberculosis, RR-TB=rifampicin resistant tuberculosis, MDR-TB=multi-drug resistant tuberculosis, XDR-TB=extensively drug resistant tuberculosis)

Drug resistant tuberculosis (DR-TB) is a major public health concern due to the high mortality, cost of treatment, the chronic and infectious nature of the disease, and risk of transmission to the public including the health care workers (6). Thus, the timeous diagnosis of drug resistance and commencement of appropriate therapy as soon as possible, is of utmost importance in reducing transmission, optimising the chances of treatment success and limiting the development of further drug-resistance (7).

In 1996, the National Department of Health (NDoH) of South Africa established the National Tuberculosis Programme (NTP) (Table 2). Over the last two decades the NTP has evolved to include the integration of TB and human immunodeficiency virus (HIV) services, the management of MDR-TB and decentralization and community-based care of MDR-TB services (4, 8, 9).

The Stop TB Strategy was launched by the WHO in 2006 to reduce the burden of TB in alignment with global targets. Governments the world over committed to its key principles in the aim of reducing the TB burden worldwide (10). In 2007, South Africa introduced the TB infection control programme that provided for administrative and environmental controls and the use of personal protective equipment during the management of TB in primary health care and community settings (11). In 2008, the WHO launched the 'STOP TB Policy' which emphasized the role of public health systems in strengthening national TB programmes (12). To cement these principles in TB management in South Africa, the NDoH updated the National Tuberculosis Management Guidelines in 2014 (13). In 2015, the United Nations released the Sustainable Development Goals (SDGs). One of the goals of the SDGs is to have a world free of TB, with zero deaths and suffering due to TB by 2030 (14). In an attempt to achieve these goals, the "End TB strategy" 90-90-90 targets were introduced. These targets include that 90% of all people who need TB treatment are diagnosed and receive the appropriate therapy (including first-line, second-line and preventative therapy, as required), 90% of all those in key and vulnerable populations are diagnosed and receive the appropriate therapy and that a treatment success rate of 90% is achieved for all those diagnosed with TB by 2030 (15). In response, South Africa adopted the End TB targets as part of the national strategy to address the burden of TB in South Africa (1, 4).

In the last decade, a number of bold decisions have been taken by the National Department of Health in an attempt to address the burden of TB in the country. These include the introduction of rapid diagnostic tests for drug susceptible and DR-TB, new pharmaceuticals for the treatment of MDR- and XDR-TB, as well as decentralising the management of MDR-TB to district level hospitals and primary health care clinics in an attempt to provide care and support for patients closer to their homes (13). Furthermore, in 2015, South Africa launched a comprehensive TB screening and testing campaign, focussing on key vulnerable groups, such as inmates in correctional facilities, mineworkers and children (11, 16).

In 2010, of the 7386 cases of laboratory confirmed MDR-TB, only 5402 (73%) patients started treatment for MDR-TB (17). The 27% of patients diagnosed with MDR-TB but never initiated on treatment was exacerbated by the length of time taken to receive laboratory results (17, 18). In the delay period, many rural patients were lost to the system as they may have died, or remain untreated (17, 18). In 2015, a third of all new patients with confirmed TB, as well as those previously treated with TB, underwent drug susceptibility testing for rifampicin, whereas the rest were treated based on smear-positive results alone (6).

Since the inclusion of the GeneXpert MTB/RIF assay (Cepheid Inc.) (Xpert) platform in the South African TB diagnostic and management algorithm, 10,566,489 specimens were processed between 2011 and 2017. Of those, 10.37% were diagnosed positive for TB and 6.54% of the positive TB specimens were RR-TB (19). With the introduction of Xpert, the number of diagnosed RR-TB cases increased from 7386 in 2010 to over 10 000 in 2011, and approximately 20 000 in 2015 (17). Despite these improvements in DR-TB detection rates, there remains a significant gap between diagnosis and initiation of appropriate treatment.

1st line Line Probe Assay (Hain MTBDRplus) (LPA) was introduced in 2008 to diagnose rifampicin (RIF) and isoniazid (INH) resistance. After the introduction of Xpert, it was used to confirm RIF resistance and determine INH susceptibility, as Xpert only detects RR. The 1st line LPA was performed on culture isolates as per the WHO protocol and national diagnostic algorithm (20), and in 2017 the 2nd line LPA, which detects fluoroquinolone and injectables resistance, was introduced to shorten the time to the diagnosis of XDR-TB (21).

1997	Phased implementation of directly observed therapy, short course (DOTS), establishment of demonstration and training districts (DTD).
1999	Introduction of fixed-dose combination drugs Establishment of TB and HIV pilot districts
2000	MDR-TB guidelines endorsed, establishment of MDR-TB treatment facilities.

	Four-drug fixed-dose combination tablets introduced
2001	National Drug Resistance Survey
2002	Launch of the Medium-Term Development Plan (MTDP), 2002 - 2005 Guidelines for isoniazid preventative therapy (IPT) for tuberculin skin test (TST)-positive, HIV-infected persons
2003	TB declared an emergency and TB crisis plan launched Electronic TB register introduced
2005	Minister of Health signs 'Declaration of TB as an emergency in AFRO (African regional office) region'
2006	Development of MDR-TB and XDR-TB action plan
2007	Launch of the National TB Strategic Plan 2007 - 2011 Development of infection control guidelines for TB
2008	Introduction of 1st line LPA (Hain MTBDRplus) as a rapid test for MDR-TB (performed on culture isolates)
2009	'Health in South Africa' series published in *The Lancet*, including recommendations for TB/HIV WHO review of the NTP
2010	6-month IPT for all HIV-infected persons, regardless of TST status Anti-retroviral therapy (ART) for TB patients living with HIV with CD4+ counts <350 cells/µl
2011	Introduction of Xpert MTB/Rif as a replacement for sputum smear microscopy National HIV/TB campaign Management of DR-TB policy guidelines approved Decentralised management of MDR-TB introduced
2012	SA President signs SADC declaration on 'TB in the mines' ART for all HIV-infected TB patients Streptomycin removed from retreatment regimen
2013	NDoH guidelines for managing TB/HIV in prisons issued IPT for at least 36 months for TST-positive, HIV-infected persons National drug resistance survey Independent WHO Review of NTP
2014	Updated National TB Management Guidelines

2015	Launch and roll out of mass TB screening programme
2017	Introduction of direct (performed directly on clinical samples) first and second line reflex testing using LPA

Table 2: Key Milestones in the South African national Tuberculosis programme 1997 – 2017 (8, 22)

(Definition of Abbreviations: TB=Tuberculosis, HIV=Human Immunodeficiency virus. DR-TB=drug resistant tuberculosis, RR-TB=rifampicin resistant tuberculosis, MDR-TB=multi-drug resistant tuberculosis, XDR-TB=extensively drug resistant tuberculosis, LPA=Line probe assay, NTP=National Tuberculosis Programme, WHO=World Health Organization)

The management of DR-TB in South Africa is highly dependent on timeous laboratory results and the consequent rapid initiation of treatment to prevent further transmission, as well as promote adherence to the treatment regimen to reduce resistance development. In July 2017, following the WHO recommendation, the National Health Laboratory Service (NHLS) introduced direct sputum testing using LPA, for both first and second line anti-TB drugs, for all Xpert MTB positive and RIF resistant samples to accelerate DR-TB diagnosis (7, 20). This would ostensibly help to achieve the South African DR-TB Policy Guidelines recommendation of treatment initiation within 5 days of diagnosis (23).

1.2. DR-TB Diagnostics in South Africa

Historically, TB control programmes have relied on passive case finding whereby patients with TB symptoms present themselves to primary health care facilities. However, to meet the targets of the End TB Strategy, new strategies encouraging active case finding by screening high risk populations through community and primary health care facility interventions have been introduced (24).

Prior to 2011, the primary diagnostic method for TB in South Africa, was smear microscopy, and this has since been replaced by Xpert (10). Acid-Fast Bacilli (AFB) smear microscopy is still an important part of the management of MDR-TB in South Africa, as it is used to estimate bacillary load in sputum and thus identify patients most likely to transmit TB and also used to monitor sputum conversion as an indication of whether the patient is responding to treatment. The advantages of smear microscopy are that it is inexpensive, does not require advanced infrastructure, which is important in limited resource settings, and most importantly yields same day results. The limitations of smear microscopy include its decreased sensitivity, especially in patients with a bacillary load of less than 5000 AFB/ml of sputum, lower sensitivity in HIV co-infected patients, non-differentiation between various mycobacteria and its non-differentiation between live and dead bacilli (25). Furthermore, AFB smear microscopy cannot detect drug resistance (13, 25, 26). Despite these limitations, AFB smear microscopy remains a component of TB (susceptible and DR-TB) management and treatment monitoring.

Xpert is an automated, polymerase chain reaction (PCR) diagnostic tool for the simultaneous detection of *Mycobacterium tuberculosis* (*MTb*) and RR-TB. In 2010, the WHO endorsed Xpert as a replacement

for smear microscopy and South Africa adopted this policy change in 2011 (27). The test has a short turnaround time (2 hours) and it is specific for *MTb* (28). Furthermore, it can be used on a variety of specimens including cerebrospinal fluid, gastric and lymph node aspirates and tissue. There is less opportunity for human error and contamination, as the testing is fully automated within a closed cartridge (10). Although the infrastructure and initial technological outlay is expensive, cost-effectiveness studies have shown that, considering the cost of TB treatment, potential transmission risks in untreated and incorrectly treated patients, the cost of the Xpert is justified (29, 30). This diagnostic tool, however, is limited to diagnosis and cannot be used in monitoring treatment. A further complication of the Xpert is how to manage discordant results between genotypic and phenotypic methods, commonly a consequence of genotypic gene expression without phenotypic resistance (31). Furthermore, Xpert can only detect RR and thus cannot diagnose MDR-TB and XDR-TB, but it is used as a proxy for MDR-TB.

Although South Africa consumes the highest quantities of Xpert cartridges worldwide, a number of health system factors undermine the capacity of Xpert to ensure patients with RR-TB are diagnosed and started on effective treatment in the shortest possible time (32). A study performed by McCarthy, *et al.* in 2015 during the scale up of the Xpert protocol, revealed that only 14% smear positive, Xpert negative and 32% smear negative HIV-infected individuals had subsequent sputum samples cultured, which according to the diagnostic algorithm at the time, should be done for all HIV-positive individuals (33). This has implications in TB detection rates, essentially leaving a proportion of the population with a high risk of TB untested and possibly unidentified, and therefore untreated.

Xpert Ultra (Xpert Ultra) was developed to improve the sensitivity of this test in patients who are smear negative and co-infected with HIV (34, 35). It was introduced in South Africa in 2017 (36), however, a multicentre study that recruited TB infected adults from eight countries, demonstrated that this increase in sensitivity resulted in a consequent decrease in specificity (35). Despite the improvements in sensitivity, it still only detects RR-TB and thus further testing is required for XDR-TB diagnoses.

Culture is more sensitive than smear microscopy (able to detect 10 AFB/ml sputum) and is still regarded as the gold standard for TB diagnostics. It identifies between mycobacterium species, distinguishes between live and dead bacilli and allows for drug susceptibility testing (DST). In high burden countries, culture is performed on solid Lowenstein-Jensen (LJ) medium, which although has a higher sensitivity that a smear, has an extended incubation time (4 – 6 weeks). For this reason, in 2007, the WHO recommended that liquid culture be used as a standard reference for *MTb* diagnosis and DST (37). Liquid culture medium, like the Mycobacteria Growth Indicator Tube system (MGIT; Becton, Dickinson), yields results much earlier than solid culture media. Depending on the bacterial burden, a culture grown on a liquid medium can yield results within 17 – 20 days. In comparison, a culture grown on a solid medium will yield results in 42 days. In addition, a culture grown on a liquid medium will have a higher yield of bacteria (29.7%) compared to 22.8% for solid medium. Liquid culture systems

can be automated (38, 39), but are expensive and require advanced infrastructure and expertise and can take up to 42 days to obtain a result. It also requires higher levels of biosafety (10, 26). Liquid media was introduced in South Africa in 2007 after the WHO recommended its use for drug susceptibility testing in low and medium income settings (40).

The Line Probe Assay is a molecular PCR-based diagnostic technique that simultaneously determines rifampicin (RIF) and isoniazid (INH) resistance in its first line assay, and fluoroquinolones (FLQ) and second line injectables (INJ) in the second line assay. This assay was initially introduced as 1st line only, for indirect testing on cultured isolates and therefore delayed MDR-TB confirmation (41). This assay is currently approved for testing patient samples directly, thus eliminating the time to wait for culture to become positive. The LPA has a better sensitivity than microscopy, between 63% and 100% for pulmonary and extrapulmonary cases, respectively, opposed to 46.5 % and 12% respectively for microscopy (7) (Table 3). However, the LPA cannot be used for monitoring treatment, because it detects DNA, whether from live or dead bacilli, and it requires a high level of laboratory expertise and infrastructure (10). The laboratory turnaround time for direct LPA performed on sputum is 24 – 48 hours, opposed to indirect LPA performed on culture isolates, of which the turnaround time is 4 – 8 weeks (42, 43).

Before the introduction of the Xpert in South Africa, drug resistance testing was only conducted on patients with TB who were not responding to treatment, had MDR-TB contacts or experienced recurrent infections, whereas current protocol dictates Xpert on all suspected TB cases (23). The new algorithm for TB diagnostic and management in South Africa follows the WHO recommended guidelines (26). A standardised drug resistant TB-treatment regimen is initiated on patients with an Xpert positive and RR-TB result. As of July 2017, first and second line DR-reflex testing (using 1st and 2nd line LPA) is performed directly on patient's sputum samples to confirm rifampicin resistance and establish any further drug resistance (Figure 1 and 2). If further drug resistance is found, the treatment regimen is altered accordingly to ensure the patient receives the most appropriate treatment (26, 44). The direct 1st and 2nd line LPA detects resistance to first and second line drugs within 5 days (stipulated as 28 days in the national guidelines to allow for delays in turnaround times), allowing patients to be initiated on appropriate treatment more quickly, thereby reducing transmission time, and ultimately the burden of DR-TB (45). Often patients with DR-TB are admitted for 4 – 6 weeks for the initiation period and after discharge, return for monthly assessments, which includes monitoring the response to treatment with acid-fast bacilli (AFB) smear microscopy and DST culture, documenting the development of adverse events and the issuing of further medication (26).

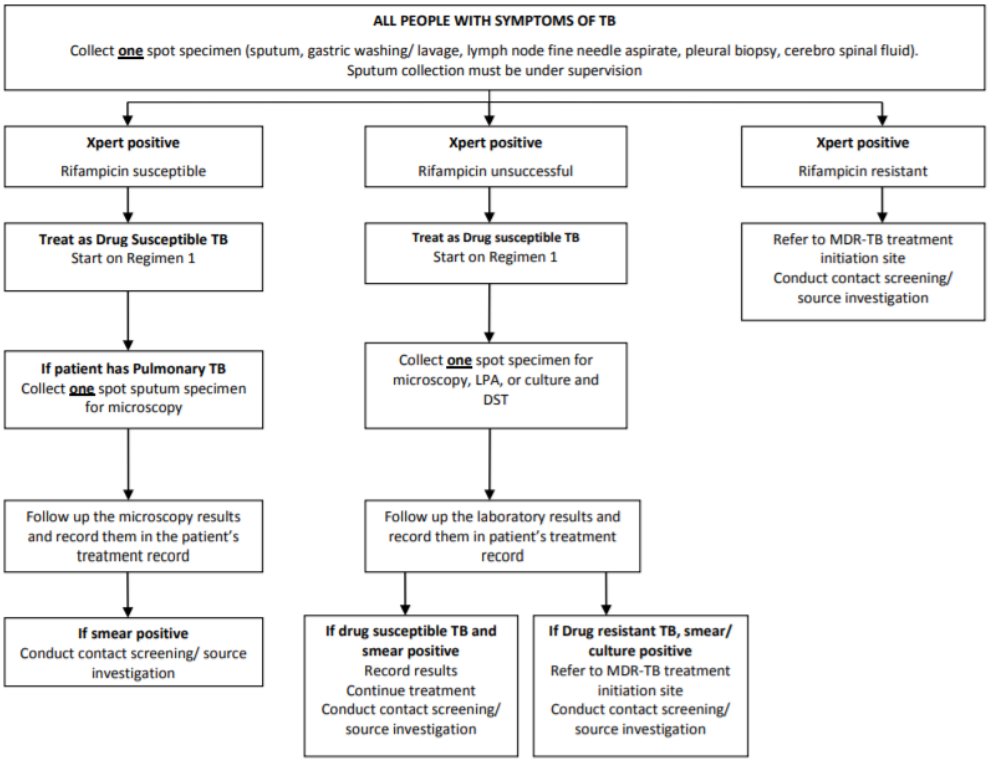

Figure 1: Xpert based tuberculosis diagnostic algorithm in South Africa 2014 (13)

(Definition of abbreviations: Xpert= GeneXpert MTB/RIF assay (Cepheid Inc.))

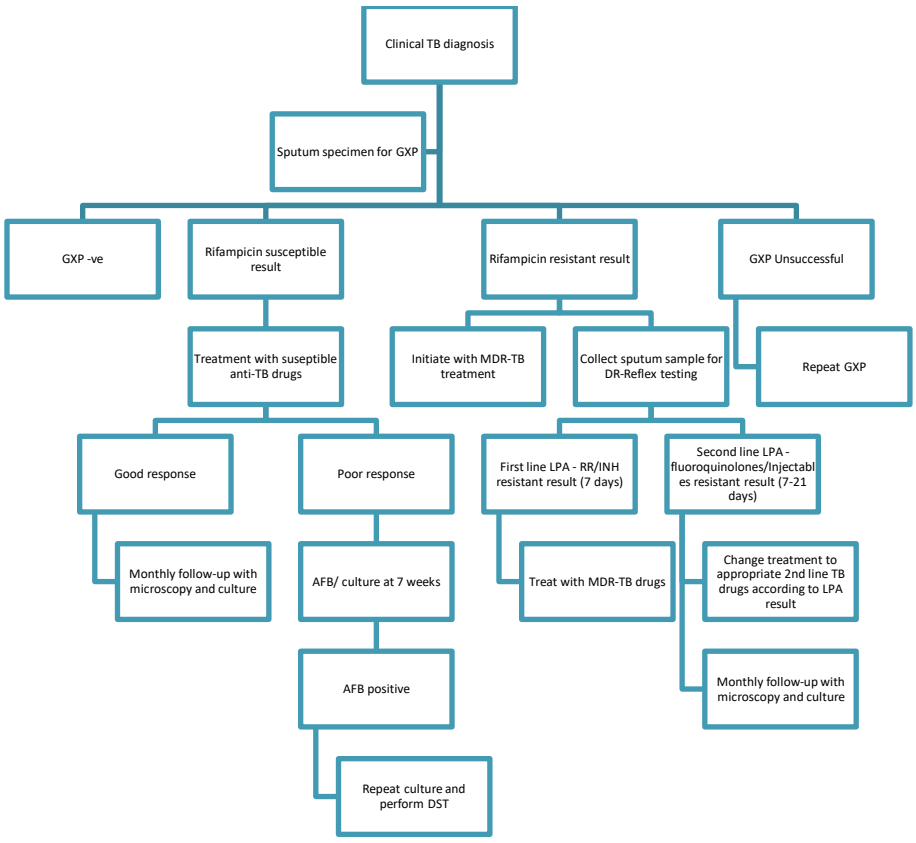

Figure 2: Flow diagram depicting the management of TB in South Africa 2017 (8)

(Definition of abbreviations: GXP=GeneXpert MTB/RIF assay (Cepheid Inc.), DR=drug resistant, INH=isoniazid, LPA=Line probe assay, MDR-TB= multi-drug resistant tuberculosis)

Evaluation of Xpert implementation has shown that, although there have been positive improvements in TB diagnosis, the implementation of Xpert did not produce the expected improved treatment outcomes due to health system issues (46). One of the assumptions prior to the implementation of Xpert was that earlier diagnosis of DR-TB would allow for earlier initiation of appropriate treatment and ostensibly reduce transmission. With the introduction of the Xpert there was an increase in the proportion of patients diagnosed with DR-TB who were initiated on treatment, from 55% in 2011 to 63% in 2013. In addition, the median time to treatment initiation decreased from 44 days in 2011 to 22 days in 2012, but only 10% of newly diagnosed DR-TB cases were initiated within the 5-day national target (47). However, a major concern was that over a third (37%) of those diagnosed with RR-TB were still not initiated on treatment (46).

The gap between the number of people diagnosed with RR-TB and those initiated on treatment is known as the treatment gap. Prior to the roll-out of Xpert, the reasons for the treatment gap of 38% included high pre-treatment mortality, long delays in receiving drug susceptibility results and the need for referral to a health facility which could initiate MDR-TB treatment. The impact of the roll-out of Xpert was not as expected and failed to reduce the time to treatment as much as was hoped (46). Xpert allowed for rapid diagnosis of RR-TB and initiation on MDR-TB treatment much earlier. However, as the diagnosis of additional resistance to injectables and fluoroquinolones was still delayed as this required the growth of positive cultures to allow for phenotypic 1st and 2nd line DST. This provided a catalyst for NDoH to introduce faster second-line diagnosis as this would facilitate more rapid initiation of appropriate treatment.

		***MTb* Detection**		**RIF resistance**	
Method		**Sensitivity (%)**	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**
AFB Smear (48)		53.0	100	N/A	N/A
Culture		93.5	100	100	100
Xpert (34)	Total	65	98	95	98

Method		*MTb* Detection		RIF resistance	
		Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
	Smear positive	83	98		
	Smear negative	46	98		
Xpert Ultra (34)	Total	76	96	95	98
	Smear positive	88	96		
	Smear negative	63	96		
LPA (49)	Total	90	95	88	95
	Smear positive	95	100	94	100
	Smear negative	75	94	73	94

Table 3: Comparison of the sensitivity and specificity of AFB, Culture, Xpert and Xpert Ultra for the detection of *MTb* and RIF resistance (34, 48-51)

(Definition of abbreviations: AFB=acid fast bacilli, Xpert=GeneXpert MTB/RIF assay, Xpert Ultra= GeneXpert Ultra MTB/RIF assay, LPA=Line probe assay, *Mtb=Mycobacterium tuberculosis*, RIF=rifampicin)

1.3. Do Molecular Techniques Reduce to the Time to Treatment Initiation?

A Latvian study demonstrated a reduction in time to treatment initiation from 40 to 7 days for first time TB cases in an observational cohort study performed between 2009-2012 after the implementation of Xpert (52). In South Africa, Naidoo et al, showed that the introduction of direct sputum testing with Xpert reduced the time to MDR-TB treatment commencement from 43 days to 17 days (53). A study performed at a specialised TB treatment facility in KwaZulu-Natal, found that the introduction of Xpert reduced the time from sputum collection to treatment initiation from 92 days to 20 days (45).

The success of the implementation of Xpert in reducing the treatment initiation delay informed the decision to implement first and second line DR-TB reflex utilizing direct LPA in South Africa. A retrospective, cohort study in Delhi, India investigated the impact of the introduction of 1st line LPA, performed on culture isolates, on time to treatment initiation of MDR-TB treatment, following the

revision of their National Tuberculosis Control Programme in 2011. The median time from diagnosis to MDR treatment initiation was significantly reduced from 157 days to 38 days (*p=0.001*). The study also revealed lower losses to follow up during treatment from 39% before LPA implementation to 12% after the implementation of LPA (54). A South African study to investigate the impact of the implementation of 1st line LPA (performed on culture isolates), as a replacement of conventional 1st line DST, on the mean time to treatment, revealed that the mean treatment commencement time significantly decreased to 62 days with LPA, compared to 78 days with DST (*p=0.045*) in a demonstration study (55). An operational study further tested the mean treatment commencement times in a rural TB hospital, which showed and improvement to 55 days with LPA conducted on isolates, opposed to 80 days with conventional DST (56). These studies demonstrated that 1st line LPA conducted on culture isolates improved the mean time to treatment initiation. A study performed in a high throughput laboratory in Cape Town demonstrated that the implementation of LPA (performed on culture isolates in this case) reduced the laboratory turnaround time to 3 days (IQR 2 – 5) with LPA, from 45 days (IQR 27 – 122) with phenotypic DST (*p<0.001*). The research further showed that the time to reporting of 2nd line LPA results was 31 days (IQR 13 – 82) (57).

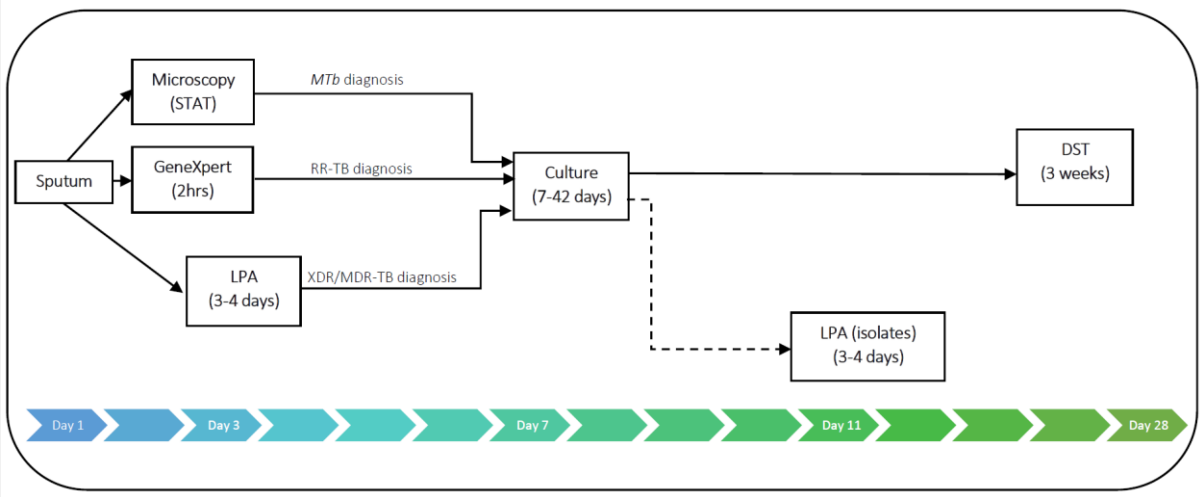

Figure 3: Accumulative turnaround time for Culture, DST, LPA, Xpert and AFB microscopy

(Definition of abbreviations: *Mtb=Mycobacterium tuberculosis*, LPA=Line probe assay, RR-TB=rifampicin resistant tuberculosis, XDR/MDR-TB diagnosis=extensively-/multi-drug resistant tuberculosis, DST=drug susceptibility testing)

As shown in the diagram above (Figure 3), the direct (performed directly from clinical samples, as opposed to culture isolates) LPA reduces the time to detecting resistance to both 1st and 2nd line TB drugs. This allows for significant reduction in time to drug susceptibility testing so that appropriate treatment can be initiated timeously.

1.4. Does Reduced Time to Treatment Initiation Affect Treatment Outcomes?

It was assumed, that by reducing treatment initiation delay and starting treatment sooner, treatment outcomes would improve.

A Russian study compared the impact of a direct LPA-based diagnostic algorithm on time to treatment initiation and treatment outcomes for MDR-TB patients, with a culture-based algorithm. The results demonstrated a reduction in the time to treatment initiation with the implementation of LPA of 50 days (*p=<0.001*) compared to liquid culture and 66 days (*p<0.001*) compared to solid culture media. The LPA-based algorithm had a 65.2% treatment success rate, opposed to the culture-based algorithm which had a success rate of 44.8%. There was also a lower mortality with the LPA-based algorithm group (7.6%), as compared to the culture-based algorithm group (15.9%). The LPA cohort showed overall more favourable treatment outcomes compared to the culture-based algorithm (*p=0.003*) (58).

A study in China investigated whether there was an association between time to treatment initiation and treatment outcomes. The results showed that those patients with favourable outcomes were initiated on treatment a median time of 172 days after diagnosis, as opposed to those with unfavourable outcomes, who were initiated a median of 190 days after diagnosis. In addition, they reported that treatment initiated within 60 days of diagnosis were associated with favourable outcomes (odds ratio=2.56, 95%CI=1.22 – 5.36) (59).

A South African study assessing the clinical impact of the introduction of Xpert in MDR-TB patients, found that although there was significant improvement in the time to treatment initiation, it did not translate into more favourable patient outcomes (45). Churchyard *et al*. found that Xpert did not reduce mortality at 6 months, as compared to sputum microscopy, and suggested that better treatment outcomes could be obtained by addressing health system issues and improving linkage to care (60).

A third South African study reported a median time to treatment initiation of 11 (range=0 – 180) days, but the reduced time to treatment initiation was not associated with an increase in favourable treatment outcomes (*p = 0.795*) (61).

As the direct 2nd LPA has only been introduced recently, there is no data as yet available documenting whether it impacts on treatment outcomes.

1.5. Comparison of LPA with Phenotypic methods

A study to assess the correlation between genotypic and phenotypic testing for rifampicin resistance was conducted in Haiti (62). In this study 89.5% of the genotypic (Xpert and LPA) results were confirmed RIF-resistant by DST. The remaining 10.5% were diagnosed as RIF-resistant by molecular methods, but RIF-susceptible by DST. This discrepancy was investigated and it was found that the manifestation of the genotypic *rpoB* mutations did not result in phenotypic expression of rifampicin

resistance in every case, as they contained a silent mutation (not phenotypically expressed) (62). These silent mutations do not impede the action of rifampicin, as they do not result in structural changes in DNA-dependent RNA polymerase (63). Unfortunately, this could result in an incorrect diagnosis of MDR-TB (62). A Chinese study suggested that there is an epistatic interaction of mutations relating to RIF resistance and strain fitness, resulting in the presence of *rpoB* mutation. These mutations, whether expressed or not, play a role in the increased transmission of DR-TB as they are associated with the development of secondary mutations that impact positively on strain fitness (64).

Research conducted in India compared direct LPA with solid culture DST methods for the diagnosis of MDR-TB on culture positive samples. Overall, LPA detected MDR-TB 96% of the time, as compared to conventional DST using solid culture. Compared with solid culture, the sensitivity and specificity of LPA was 98% and 99% respectively for the detection of RIF-resistance; 92% and 99% respectively for INH resistance; 97% and 100% respectively for the detection of MDR-TB. The LPA laboratory turnaround time was 48 hours. Thus, LPA proved successful in the early diagnosis of mono-resistance to INH and RIF in high-burden countries (65).

2. Research Gap and Questions

The current literature demonstrates that molecular techniques effectively reduce the time to treatment initiation, but only a few studies document a correlation between treatment initiation and treatment outcomes. A systematic review published in 2016 stated that there was no published evidence to support the assumption that treatment initiation delay in MDR-TB cases leads to poor treatment outcomes (66). This review had limitations including the differences between groups of patients, and thus the inability to effectively compare them. In addition, differences in treatment outcomes could not be attributed to decreased time to treatment initiation. Furthermore, there are other processes and factors that could affect both time to treatment initiation and treatment outcomes. From the perspective of the health care provider these include health system changes between the periods, improved anti-TB medication and decentralisation of TB care. There are also patient-level factors which affect both time to treatment initiation and treatment outcomes. These include the accessibility of the health facility, family support and household responsibilities. Further research is necessary to compare the impact of 1st and 2nd line DR-TB testing on treatment outcomes to exclude the impact of other factors on treatment outcomes.

It is imperative to discern whether the algorithm is being correctly implemented and if not, what challenges are hindering the implementation. In this particular instance, evidence concerning TB diagnostics is critical for the development of evidence-based protocols and ultimately to contribute positively to reducing the burden of TB (67). This reinforces the need for this study.

There is a lack of evidence regarding the effectiveness of existing guidelines, thus the knowledge gap needs to be filled with empirical research which demonstrates the effectiveness of public health policies (68). This study will investigate the following null hypotheses:

2.1. There is no significant delay between the diagnosis and appropriate treatment initiation in DR-TB infected patients in the Umzinyathi district. A significant delay is defined as > 5days, as per the National Guidelines.

2.2. The delay in the initiation of appropriate treatment is not significantly different after the implementation of 1st and 2nd line LPA in DR-TB reflex testing, as compared to the appropriate treatment initiation delay before the introduction of 1st and 2nd line LPA in DR-TB reflex testing.

2.3. There is no significant improvement in treatment outcomes after the implementation of DR- reflex testing.

2.4. There is no significant difference between 1st and 2nd line LPA results and phenotypic DST results

3. Aims and Objectives

The aim of this study was to assess the impact of the introduction of direct 1st and 2nd line DR-TB reflex testing on time to diagnosis, time to treatment initiation and treatment outcomes in patients with DR-TB.

The objectives of this study are:

3.1. To document the delay in initiation of appropriate treatment and the treatment outcomes in patients with DR-TB before and after the introduction of DR-TB reflex testing.

3.2. To compare the treatment initiation delay and treatment outcomes before and after the introduction of DR-TB reflex testing.

3.3. Analyse first and second line LPA results and compare these to phenotypic DST results.

3.4. To document operational challenges associated with the implementation of DR-TB reflex testing in routine health services.

4. Contextual Framework

In this study, we evaluated the introduction of the direct 1st and 2nd line LPA tests in a district hospital in a rural district in KwaZulu-Natal to determine the time from sputum collection to laboratory results for the 1st and 2nd line LPA tests, whether the algorithm has been effective in such a setting, and the

time taken for patients to be initiated onto appropriate treatment. Furthermore, to investigate if patient outcomes have significantly improved with the introduction of the 1st and 2nd line LPA. Additionally, challenges both at clinic, hospital and laboratory level were explored to thoroughly investigate and evaluate the effectiveness of the roll out of this diagnostic program.

This research has importance in the field of medical microbiology and public health. The study serves to assess the value and effectiveness of the roll out of DR-reflex testing protocol in a rural hospital. Furthermore, the study explored the challenges experienced with the roll-out.

By comparing treatment initiation delay and outcomes prior to the implementation of DR-reflex testing and after the roll-out, we were able to evaluate whether direct 1st and 2nd line LPA has indeed improved time to appropriate treatment and improved patient outcomes.

The study has provided data that will inform future implementation of diagnostics tests.

CHAPTER 2

5. Methodology

5.1. Study Design

This study was an observational, retrospective cohort study.

5.2. Setting

South Africa has a district health system with 52 districts across all 9 provinces. Umzinyathi, one of KwaZulu-Natal's 11 districts, was selected for the study. The estimated population of Umzinyathi is 555 485, with a TB incidence of 250 per 100 000, MDR-TB prevalence of 26.8 cases per 100 000 and an HIV prevalence of 6.7% (21, 24, 69, 70). The DR-TB programme execution and monitoring are well implemented with an overall treatment success rate of 89%,death rate of 9.8% and rate of loss to follow-up of 0.4% in 2017 (71, 72). Greytown hospital, MDR-TB unit, which was decentralized in 2017, started treating DR-TB patients from the Umzinyathi district in 2008.

The study included all patients diagnosed with DR-TB, and initiated onto treatment, at the Greytown TB hospital from 1 July 2015 until 30 June 2018. The outcomes of patients were defined as the treatment outcome 9 months after treatment was initiated.

5.3. Data Collection and Analysis

A retrospective record review was performed. Data were collected and analysed to document time taken to initiate patients diagnosed with DR-TB on appropriate treatment and treatment outcomes before the introduction of direct 1st and 2nd line LPA reflex testing (1 July 2015 – 30 June 2016, referred to as cohort 1) and after the introduction of this LPA reflex testing (1 July 2017 – 30 June 2018, referred to as cohort 2).

The 1st and 2nd line LPA results were analysed and compared to phenotypic DST results for both periods (1 July 2015 – 30 June 2016 and 1 July 2017 – 30 June 2018), noting that for cohort 1, there was only the 1st line LPA, which was performed on culture isolates. The clinical and laboratory challenges associated with the introduction of direct DR-TB reflex testing protocol were documented.

Retrospective data were collected from the laboratory and patient records which were accessed directly from the online NHLS TrakCare^{TM} Laboratory Information System and the Electronic Drug-resistant TB Register (EDR Web) program. Data were captured into a Microsoft Excel 365 spreadsheet (Microsoft Corporation, USA), coded and statistical analysis conducted.

The Table 4 below outlines the data that were collected from the EDR, as well as descriptions and definitions of the terminology used.

Information type	Analysis Parameter	Description
Patient-level Characteristics	Age	Age of the patient at the start of treatment
	Gender	
	Medical history	Type of TB
		Treatment information
	Patient Category	New – Has never been treated for TB or have taken anti-TB drugs for less than 1 month
		Recurrent – Has previously been treated for TB and were declared *cured* or *treatment completed* at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB
		TF1 –Treatment after failure of first line drugs: Have previously been treated for TB with 1st line anti-TB drugs and whose *treatment failed* at the end of their most recent course of treatment.
		TAL – Treatment after loss to follow up
	Previous Drug History	New Case – Has never been treated for TB or have taken anti-TB drugs for less than 1 month.
		PT1 – Previously treated with 1st line anti-TB drugs
		PT2 – Previously treated with 2nd line anti-TB drugs
Details of TB Management	Date of laboratory diagnosis	
	Xpert sputum sample date and results	

Information type	Analysis Parameter	Description
	Laboratory Turnaround Time	Defined as the time taken from sputum sample collection, to the availability or finalisation of the results in the laboratory.
	Date of treatment initiation	Date that the patient was initiated onto anti-TB therapy
	Time to appropriate treatment initiation	All patients diagnosed with RR-TB on Xpert were, according to country guidelines started on standard MDR-TB treatment. When further resistance was identified either by phenotypic testing or LPA, the treatment regimen would be modified to treatment appropriate to the patient's resistance pattern. Thus, the time to appropriate treatment is defined as the time from sputum sample collection, to the date of initiation of therapy based on the above definition. If treatment was further changed due to results of laboratory test, the date of last treatment initiation was used as the date of appropriate treatment.
	Treatment initiation delay	Time between availability of laboratory results and initiation of treatment
	Date of 1st and 2nd LPA sputum sample and result	
	DR-Reflex (LPA) test results	First line LPA results
		Second line LPA results
	Drug treatment and any changes in treatment	Appropriate treatment is defined as the treatment effective against the patient's specific resistance pattern i.e. treatment initiated after drug susceptibility testing and initiated on MDR/XDR drug regimen
	Diagnostic test used as basis for treatment initiation	Defined as the diagnostic test likely to be the motive for which treatment was initiated – taken as the diagnostic test result available closest to date of treatment start or change. Recorded as

Information type	Analysis Parameter	Description
		clinical indication if no diagnostic test was performed within 30 days prior to treatment initiation
	Phenotypic testing: DST/ culture date of sputum sample and results	
	Comparison of molecular and phenotypic laboratory results	Comparison of results from Xpert, LPA and DST for the same patient
Treatment outcomes (23)	Cured	A patient who has had a TB culture conversion, received treatment for a total duration of >9 months, has had at least 3 consecutive negative TB cultures during continuation phase (at least 30 days apart) and there is no evidence of clinical deterioration
	Treatment completed	A patient who has had TB culture conversion, received treatment for >9 months, has less than 3 consecutive negative TB cultures during continuation phase (at least 30 days apart) and there is no evidence of clinical deterioration
	Loss to follow up	A patient whose treatment was interrupted for two or more consecutive months for any reason without medical approval
	Treatment failure	A patient failed to undergo culture conversion by month 4 or two or more of the five cultures recorded in the final 6 months of therapy are positive, and the patient's clinical condition is deteriorating, or if a clinical decision has been made to terminate treatment early or to change the treatment regimen by adding more than two medicines due to poor clinical and/or radiological response or adverse events.
	Died	A patient who dies from any cause during the course of DR-TB treatment

Information type	Analysis Parameter	Description
	Transferred out	A patient who has been referred from the facility to another facility in another district, province or country, for whom the treatment outcome is not known
	Moved out	A patient who has been referred from the facility to another facility in the same district, province or country, for whom the treatment outcome is not known.
	Not specified	A patient recorded in the DR-TB register who does not have the necessary recorded data to enable classification of outcome any other category. They may be continuing extended treatment due to non-adherence or complications.
Successful vs. Unsuccessful outcomes	Successful outcome	Includes: Cured and treatment completed treatment outcomes
	Unsuccessful outcome	Includes: Died, treatment failed and toss to follow up treatment outcomes

Table 4: Data and definitions thereof included in the study

(Definition of abbreviations: TB=Tuberculosis, RR-TB=Rifampicin resistant Tuberculosis, MDR-TB=multidrug resistant Tuberculosis, XDR-TB=Extensively drug-resistant Tuberculosis, Xpert=GeneXpert MTB/RIF assay, LPA=Line probe assay, DST=Drug susceptibility testing)

For the analysis of diagnostics (assessment of the performance of Xpert, 1st line and 2nd line LPA compared to phenotypic DST), data were captured from the NHLS TrakCare™ Laboratory Information System. This included the results of the diagnostic testing performed, as well as dates of sputum collection, dates of test and date of result review for all tests, on all patients, initiated within that period

5.4. Statistical Analysis

The data were analysed using the SPSS statistics (IBMv25) program.

Descriptive statistics were used to describe categorical, demographic and clinical characteristics as some of the data were not normally distributed, thus nonparametric statistical analysis was applied. A p-value of <0.05 was considered significant.

Logistic regression was used to assess the effect of risk factors on treatment outcomes.

Sensitivity, specificity, prevalence and predictive values were calculated to compare the performance between diagnostic tests. The Cohens Kappa was used as a measure of agreement between tests and interpreted as follows:

- < 0 Poor agreement
- 0.0 – 0.20 Slight agreement
- 0.21 – 0.40 Fair agreement
- 0.41 – 0.60 Moderate agreement
- 0.61 – 0.80 Substantial agreement
- 0.81 – 1.00 Almost perfect agreement (73)

5.4.1. Sample size and statistical power

Using the G*Power (v3.1.9.2) statistical program, the required sample size for comparing two independent groups is 88 in each group. Thus, the sample size in the time period 1 July 2015 to 30 June 2016 is satisfactory at 141 and the sample size in the time period 1 July 2017 to 30 June 2018 is 102 which complies with the statistical limitations (Appendix G).

5.4.2. Ethical Considerations

As the study used retrospective data, patient informed consent was not necessary and ethics approval was obtained from the BioMedical Research Ethics Committee of the University of KwaZulu Natal BREC Reference number: BE635/18 (74). In addition, approval from KwaZulu-Natal Department of Health and Umzinyathi District Health Management Team and permission to extract data from the National Health Laboratory Service databases was obtained before the study started.

To preserve patient anonymity any identifying information (name, ID number, date of birth) was kept separately from the patient data and the final database was anonymous. All electronic data was password protected and hard copy data stored in a locked cupboard.

CHAPTER 3

6. Results

6.1. Participant Characteristics

Between 1 July 2015 to the 30 June 2016, 141 patients with DR-TB were treated at Greytown MDR-TB hospital. From the 1 July 2017 to the 30 June 2018, 102 patients were treated. The mean age at treatment initiation was 37 years old for both cohorts, (SD = 12.5 and 11.1, respectively). In the 2015/2016 cohort, 88 (63.1%) new patients were initiated onto DR-TB treatment, compared to 60 (58.8%) in the 2017/2018 cohort.

In the 2015/2016 cohort, 21 (14.9%) patients were diagnosed with rifampicin mono-resistance (RMR), and 43 (30.5%) were diagnosed with RR-TB with Xpert, but INH resistance was not confirmed. A further 65 (46.1%) patients were diagnosed with MDR-TB, 5 (3.5%) had pre-XDR-TB, there was 1 (0.7%) INH mono-resistant patient and there were 6 (4.3%) patients with XDR-TB. In the 2017/2018 cohort, 19 (18.6%) of the patients were RMR, 31 (30.4%) were RR with no INH confirmation, 39 (38.2%) had MDR-TB, 3 (2.9%) had pre-XDR-TB, 1 (1.0%) patient was INH mono-resistant and 9 (8.8%) patients were diagnosed XDR-TB.

The baseline characteristics of the study participants are shown in Table 5. The two cohorts were similar with no statistical differences

		2015/2016	2017/2018	p-value
		N (%)	N (%)	
Patients initiated onto treatment		N = 141	N = 102	
Mean age at treatment start		37.2 (SD 12.5)	37.5 (SD 11.1)	0.833
Gender	Female patients	73 (51.8%)	64 (62.7%)	
Type of Drug-resistant TB	Mono-resistant (INH)	1 (0.7%)	1 (1.0%)	0.387
	Mono-resistant (RIF)	21 (14.9%)	19 (18.6%)	
	RR (INH resistance not confirmed)	43 (30.5%)	31 (30.4%)	
	MDR	65 (46.1%)	39 (38.2%)	
	Pre-XDR	5 (3.5%)	3 (2.9%)	
	XDR	6 (4.3%)	9 (8.8%)	
	New Case	88 (62.4%)	60 (58.8%)	0.631

		2015/2016	2017/2018	p-value
		N (%)	N (%)	
Previous Drug History	Previously treated with 1st line drugs	53 (37.6%)	36 (35.3%)	
	Previously treated with 2nd line drugs	0 (0.0%)	6 (5.9%)	
Pulmonary vs. Extra-pulmonary TB 2015/2016 N= 139	Pulmonary	136 (97.8%)	97 (95.1%)	0.685
	Extra-pulmonary	3 (2.2%)	5 (4.9%)	
HIV Status 2015/2016 N= 140	HIV Positive	113 (80.7%)	84 (82.4%)	0.811
	HIV Negative	27 (19.3%)	18 (17.6%)	
Anti-retroviral Therapy 2015/2016 N= 113 2017/2018 N=84	On ART	111 (98.2%)	84 (100%)	0.916

Table 5: Baseline characteristics of study participants for the period 1 July 2015 – 30 June 2016 and 01 July 2017 – 30 June 2018

(Definition of abbreviations: INH=isoniazid, RIF=rifampicin, RR=rifampicin resistant, MDR=multi-drug resistant, Pre-XDR=pre-extensively drug resistant tuberculosis, XDR=extensively drug resistant tuberculosis, HIV=human immunodeficiency virus, ART=anti-retroviral therapy)

6.2. Time to Diagnosis and Treatment of DR-TB

6.2.1. Time from Sputum Sample to Laboratory Result and to Treatment Initiation

As illustrated in Figure 4, the median time to availability of laboratory results from sputum collection (laboratory turnaround time) for Xpert was 1.0 day (IQR 0 – 1) for both cohorts (*p=0.087*). The median time to treatment initiation from sputum collection for the patients initiated on the basis of Xpert results, was 7 days for both cohorts (IQR 5 – 9 in cohort 1, IQR 5 – 12 in second cohort) (*p=0.546*). The median time to appropriate treatment initiation from sputum collection for MDR-TB patients was not significantly different between the two cohorts; 8 days (IQR 6 – 20) for MDR-TB patients in cohort 1 and 12 days (IQR 6 – 50) for MDR-TB patients in cohort 2 (*p=0.133*).

1st line LPA confirms INH resistance and thus MDR-TB; and there was a significant decrease in the laboratory turnaround time for MDR-TB diagnosis from 36.0 days (IQR 23 – 60) in the 2015/2016 cohort to 17.0 days (IQR 11 – 30) in the 2017/2018 cohort (*p=0.019*). Furthermore, the time to

appropriate MDR-TB therapy was significantly reduced from 41.0 days (IQR 35 – 61) in cohort 1, to 29 days (IQR 24 – 30) in cohort 2 (*p=0.029*) (Figure 4).

In cohort 1, XDR-TB could only be diagnosed based on phenotypic DST, which took a median of 45 days (IQR 23 – 67) to the availability of laboratory result, and a further 8 days to initiate patients onto the appropriate therapy. The introduction of 2nd line LPA in cohort 2, however, reduced the laboratory turnaround time for XDR-TB diagnosis to 21 days (IQR 12 – 50) (*p=<0.001*), and also reduced the time to appropriate XDR-TB treatment from collection of sputum specimen, from 53 days (IQR 40 – 66) in cohort 1, to 35 days (IQR 21 – 50) in cohort 2 (*p=0.033*). There was, however an increase in the delay between the availability of results and the initiation of treatment between cohort 1 and cohort 2, from 5 days to 12 days, respectively.

In cohort 1, all XDR-TB patients were initially diagnosed as having MDR-TB and started on treatment within a median of 8 days (IQR 2 – 46). On failing treatment, resistance testing was done, and they were shown to have XDR-TB and they were started on appropriate XDR-TB treatment regimens. The time to appropriate treatment initiation was taken as the time from the original MDR-TB diagnosis to the time of appropriate treatment initiation, as it was only at this point that the patients were diagnosed and treated with the appropriate 2nd line anti-TB drugs. Thus, the median time to appropriate treatment was 267 days (IQR 145 – 796) for XDR-TB infected patients in the 2015/2016 cohort. In contrast, the median time to appropriate XDR-TB treatment in the 2017/2018 cohort was 62 days (IQR 45 – 182). Thus, the time to appropriate therapy was reduced by 205 days for XDR-TB patients after the introduction of direct 1st and 2nd line LPA (*p=0.018*).

Despite laboratory results being available, Figure 4 demonstrates a delay between the laboratory diagnosis and the initiation of patients on appropriate treatment.

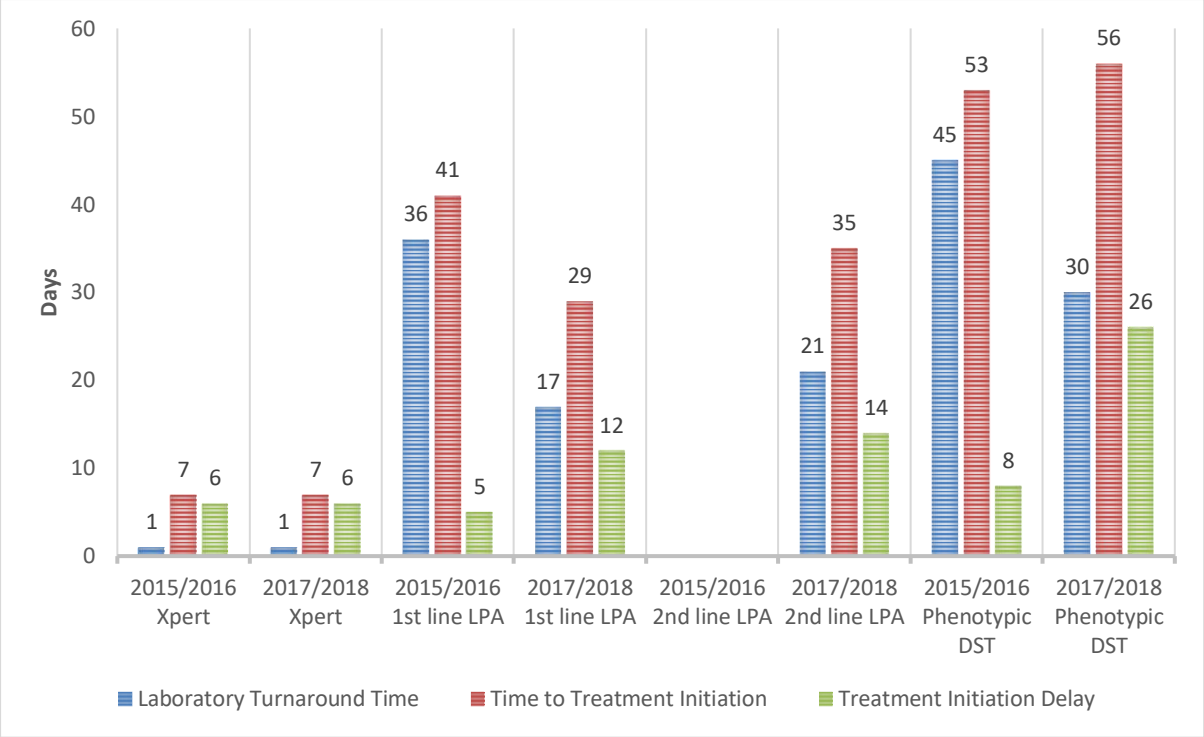

Figure 4: Illustrates the time to the availability of laboratory result (laboratory turnaround time) from sputum collection for Xpert, 1st & 2nd line LPA and phenotypic DST, compared to the time to appropriate treatment initiation from sputum collection for each diagnostic test, as well as the delay between the availability of results and treatment initiation (treatment initiation delay).

(Definition of abbreviations: Xpert= GeneXpert MTB/RIF assay, LPA=line probe assay, DST=drug susceptibility testing)

6.3. Treatment Outcomes

Figure 5 demonstrates the distribution of treatment outcomes across both cohorts. Of the 141 participants in the 2015/2016 cohort, 40 (28.4%) died, 84 (59.4%) were cured, 5 (3.5%) were lost to follow up, 2 (1.4%) completed treatment, and in 10 (7.1%) cases, treatment failed. That is, 61.0% were successful, opposed to 39.0% unsuccessful outcomes.

Of the 102 participants in the 2017/2018 cohort, 26 (25.5%) died, 55 (53.9%) were cured, 6 (5.9%) were lost to follow up, 3 (2.9%) completed treatment, 1 (1.0%) failed treatment and 11 (10%) were not evaluated (Figure 5). That is, in cohort 2, 63.7% of cases reported successful outcomes, and 36.3% had unsuccessful outcomes. There was no significant improvement reported in cohort 2 for successful outcomes (*p=0.656*).

Of the 113 (80.7%) HIV positive patients in cohort 1, 69 (61.1%) had successful treatment outcomes, compared to 46 (54.8%) of the 84 (82.4%) HIV positive patients in the cohort 2. The decrease in successful outcomes of HIV patients was not significant (*p=0.763*).

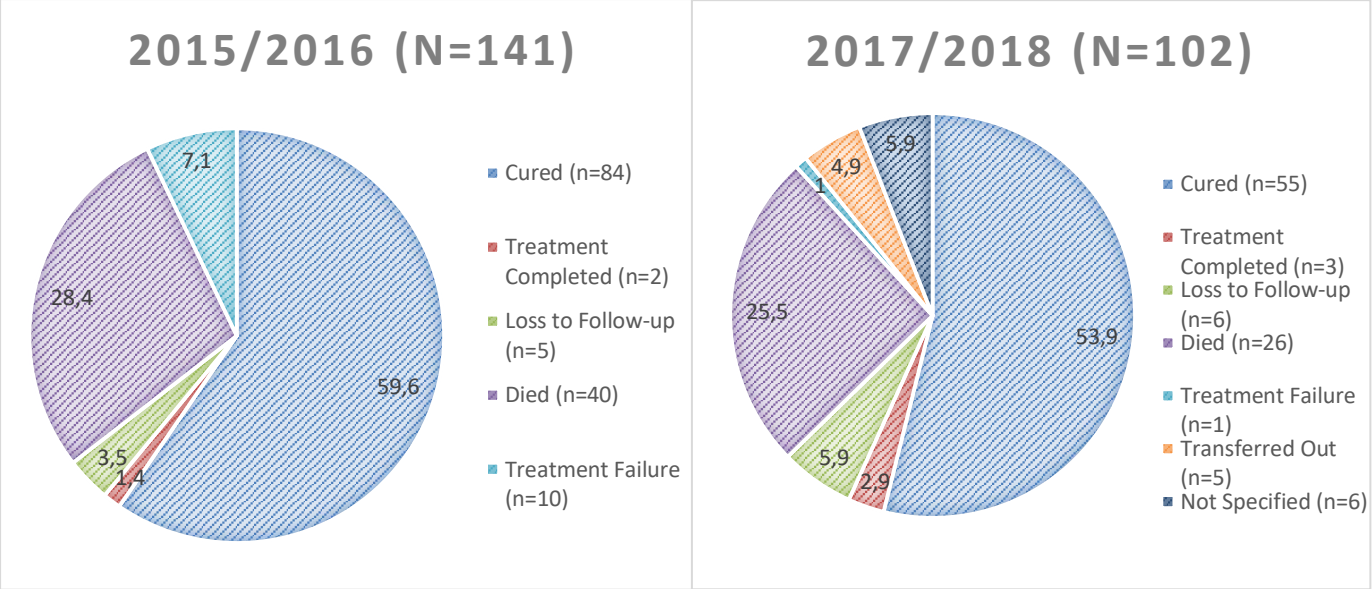

Figure 5: Percentage distribution of treatment outcomes for patients with drug resistant Tuberculosis in the Umzinyathi district during the 2015/2016 and 2017/2018 cohorts

6.3.1. Comparison of Treatment Outcomes per DR-TB Type

With regard to MDR-TB, in both cohorts the treatment cure rate was 69.2%. A smaller percentage of MDR-TB patients died in the 2017/2018 cohort, 6 (15.3%) compared to the 2015/2016 cohort, where 14 (21.5%) patients died. There were no successful treatment outcomes for XDR-TB patients in the 2015/2016 cohort, as all patients' treatment failed. In comparison, in the 2017/2018 cohort, a half of the patients had a successful treatment outcome ($p=<0.001$). In cohort 2, there was 1 (16.7%) failed treatment, 1 (16.7%) patient lost to follow-up and 1 (16.7%) death.

6.3.2. Relationship between Time to Treatment Initiation and Treatment Outcomes

Figure 6 demonstrates that in both cohorts, the most successful outcome rates occur in patients that have been placed on appropriate therapy within 15 days of sputum collection. If both cohorts were combined, the treatment success rate was 73.3% for those initiated on treatment within 15 days.

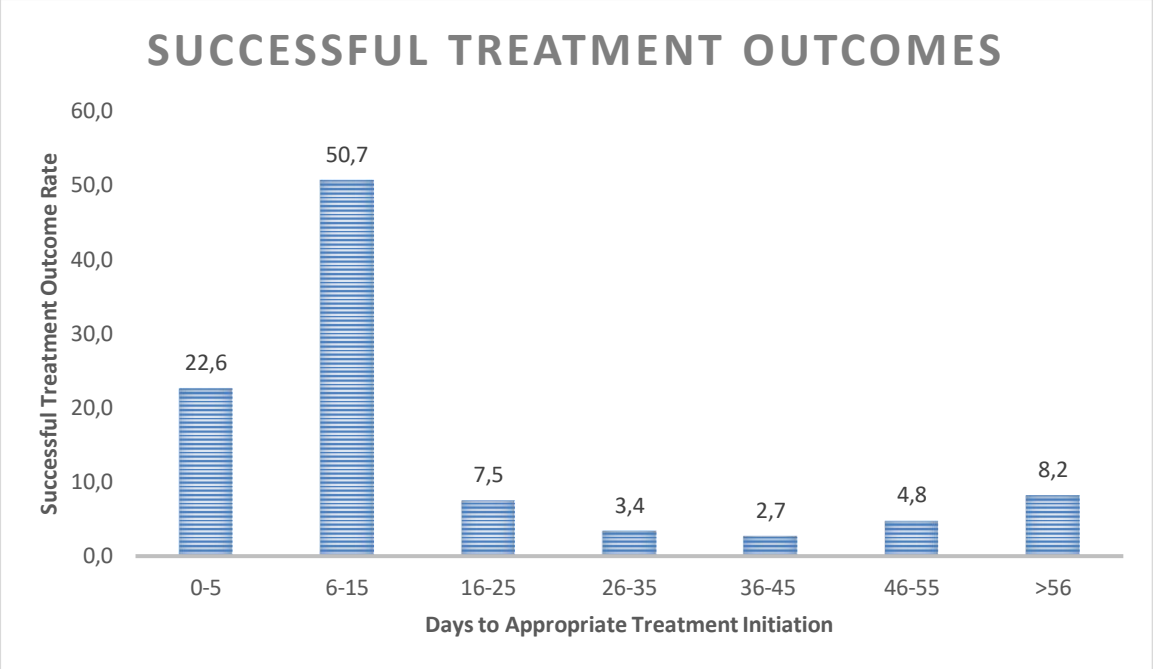

Figure 6: Successful treatment outcome rates for both cohorts (2015/2016 and 2017/2018) recorded in days to appropriate treatment initiation ranges.

6.3.3. Predictors of Successful Treatment Outcomes

In univariate analysis, patients with MDR-TB were 5.17 times more likely to have a successful treatment outcome (odds ratio (OR)=5.17, 95%CI=1.46 – 18.30, *p=0.011*) than patients with XDR-TB.

6.3.1. Changes to Treatment Regimen and its Effect on Treatment Outcomes

There were 23 (16.3%) treatment regimens that were altered in the 2015/2016 cohort, 95.7% of these regimens were altered due to subsequent laboratory tests. Whereas, in the 2017/2018 cohort, of the 18 (17.6%) treatment regimens that were altered, only 22.2% were changed due to diagnostic testing. The remaining regimen changes were due to adverse events such as ototoxicity and pre-existing hearing loss.

6.4. Comparing Phenotypic DST to Molecular Methods

6.4.1. Drug Susceptibility Results

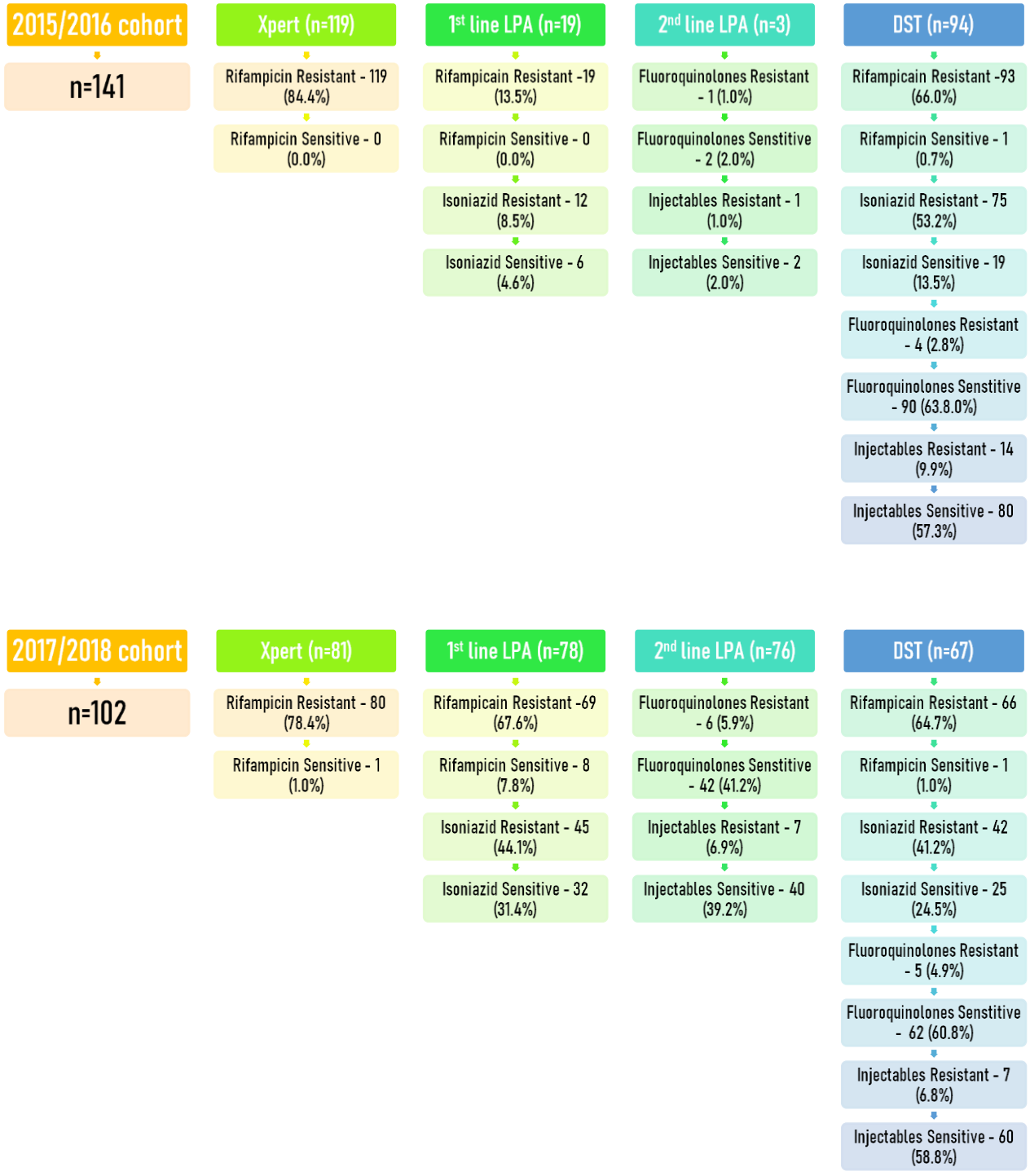

Figure 7: Drug susceptibility results across all patients initiated in the periods 1 July 2015 – 30 June 2016 and 1 July 2017 – 30 June 2018.

(Definition of abbreviations: Xpert= GeneXpert MTB/RIF assay, LPA=line probe assay, DST=drug susceptibility testing)

6.4.2. Comparison of Resistance Results across Xpert, LPA and DST

To assess the performance of Xpert and LPA in detecting antibiotic resistance, pairwise undetected results from the phenotypic DST, Xpert, 1st and 2nd line LPA were excluded. Phenotypic DST was used as the gold standard.

For Xpert, 120 results were compared with phenotypic DST for rifampicin (RIF) resistance and the Xpert results agreed with phenotypic DST 98.3% of the time, showing only 2 (1.7%) discordant results. Xpert had a sensitivity of 99.2% and a positive predictive value (PPV) of 99.2%. Specificity and negative predictive value (NPV) could not be calculated as there were no true RIF sensitive patients in either cohort. The prevalence of RIF resistance in the study sample was 99.2%. There was a poor agreement (Kappa=-0.008, *p=0.927*) between Xpert and phenotypic DST in detecting RIF resistance, but this is because specificity cannot be calculated.

Xpert and 1st line LPA were compared across 72 tests, where Xpert agreed with 1st line LPA for RIF resistance 88.9% of the time. There were 7 discordant Xpert RR results and 1 discordant Xpert RIF sensitive result (11.1%).

First line LPA results were compared with phenotypic DST for RIF resistance in 85 patients, of which the prevalence of RIF resistance in the study population was 98.8%. There were no discordant results in the dataset for 1st line LPA RR and phenotypic DST RR. There were, however, 2 discordant 1st line LPA INH-R results and 1 INH-Sensitive (S) result (3.6%). First line LPA had a sensitivity and specificity of 100.0%, as well as PPV and NPV of 100%. There is an almost perfect agreement between 1st line LPA and phenotypic DST for the detection of RIF resistance (Kappa=1.0, *p<0.001*). For isoniazid (INH) resistance, 1st line LPA was compared to phenotypic DST for 84 patients and reported a 64.3% prevalence of INH resistance in the study population. First line LPA had a sensitivity of 98.1% and a specificity of 93.3% (PPV=96.4% and NPV=96.6%). 1st line LPA showed an almost perfect agreement with phenotypic DST for the detection of INH resistance (Kappa=0.92, *p=<0.001*).

2nd line LPA agreed with phenotypic DST 90.7% of the time for FLQ-R, with 4 (9.3%) discordant results and agreed with phenotypic DST results, 92.9% of the time for INJ-R, with 3 (7.1%) discordant results. Second line LPA was compared to phenotypic DST for fluoroquinolone (FLQ) resistance for 43 patients and the prevalence of FLQ resistance in the study population was 7.0%. Second line LPA has a sensitivity of 100.0% and specificity of 90.0% for detecting FLQ resistance (PPV=42.9% and NPV=100.0%). Second line LPA showed moderate agreement with phenotypic DST for the detection of FLQ resistance (Kappa=0.56, *p<0.001*). Second line LPA was further measured against phenotypic DST for injectables (INJ) antibiotic resistance in 42 patients. The prevalence of INJ resistance in the study population was 11.9%. The sensitivity and specificity of 2nd line LPA was 100.0% and 91.9%, respectively (PPV=62.5% and NPV=100.0%). There was a substantial agreement between 2nd line LPA and phenotypic DST for the detection of INJ resistance (Kappa=0.73, *p<0.001*).

Therefore, 1st and 2nd line LPA results are not significantly different from phenotypic DST.

6.5. Challenges

6.5.1. Lack of LPA Testing

In the 2015/2016 cohort only 13.5% of the patients had 1st line LPA and 2.1% had 2nd line LPA. In the 2017/2018 cohort there was a dramatic increase in the number of LPAs performed and 88.2% had 1st line LPAs performed, and 85.3% had 2nd line LPA.

6.5.1. Inconclusive Results

Of the 11 1st line LPA tests performed in the 2015/2016 cohort, 1 (9.1%) was inconclusive, whereas in the 2017/2018 cohort, of the 254 tests performed, 42 (16.5%) were inconclusive. Of the 792 2nd line LPA, there were 151 (19.0%) inconclusive results.

CHAPTER 4

7. Discussion

In South Africa 1st line LPA was introduced in 2008 and 2nd line LPA in 2017, in our study we compared the introduction of direct 1st and 2nd line DR-TB reflex testing on time to diagnosis, time to treatment initiation and treatment outcomes in two cohorts of patients with DR-TB.cohort 1 was in 2015/2016 and cohort 2 was 2017/2018. During the 2015/2016 period, patients with RR-TB on Xpert, had a second sputum sample collected for culture, and culture positive samples had 1st line LPA done and if resistant, phenotypic DST was performed. Whereas, in the 2017/2018 period, patients with RR-TB on Xpert had a second sputum sample collected for concurrent 1st and 2nd line direct reflex testing and culture. DR-TB management and care was centralised in cohort 1, but was decentralised before cohort 2.

In our comparison, we show that the time to diagnosis decreased. The median time to availability of laboratory results from sputum collection (laboratory turnaround time) for Xpert was 1 day for both cohorts (*p=0.087*). There was a noticeable improvement in laboratory turnaround time for 1st line LPA from the 2015/2016 cohort, to the 2017/2018 cohort, of 36 days to 17 days, respectively (*p=0.019*) The laboratory turnaround time for phenotypic DST was similar across both cohorts, 53 days and 56 days, respectively (*p=0.271*). Furthermore, there was a significant decrease in time to XDR-TB diagnosis from baseline sputum sample (resistance to 2nd line drugs), from 45 days for phenotypic DST in cohort 1 to 21 days for 2nd line LPA in the cohort 2 (*p=0.033*).

In the cohort 1, patients were initially tested with Xpert, with subsequent culture and then 1st line LPA performed on positive culture isolates and subsequent phenotypic DST if indicated. 2nd line LPA was not available in cohort 1 period. Whereas, in the 2017/2018 cohort, patients were initially tested with Xpert, then DST was performed by direct 1st and 2nd line LPA performed on clinical samples. This is verified by the fact that there were similar numbers of patients tested with Xpert and phenotypic DST in both cohorts, but 1st and 2nd line LPA saw a marked increase in numbers of patients tested in cohort 2. This effectively illustrates the change in the diagnostic algorithm to include 1st and 2nd line LPA as a reflex test for DR-TB. Furthermore, it is demonstrated that the change in the algorithm impacted the laboratory turnaround time for diagnostic tests, specifically 1st and 2nd line LPA.

Our time to treatment initiation results for XDR-TB patients were consistent with the findings of Barnard, *et al*. in their investigation of 2nd line resistance diagnostics to determine the impact of LPA on the laboratory turnaround time. The study found that the time from receipt of sample specimens to reporting of 2nd line resistance results was 31 days with LPA, opposed to 45 days with culture-based DST. Similarly, our study showed time from sputum sample to availability of laboratory results was 35 days for 2nd line LPA, compared to 53 days with phenotypic DST (57).

In our comparison of time to appropriate treatment initiation, the median time to appropriate treatment initiation for MDR-TB patients was not significantly different between the two cohorts; 8 days for

MDR-TB patients in cohort 1 and 12 days for MDR-TB patients in cohort 2 (*p=0.133*). For XDR-TB patients, however, the time to appropriate treatment was significantly reduced from 267 to 62 days, between the two cohorts (*p=0.018*). However, the time to treatment initiation for patients with XDR-TB, was further reduced to 35 days with those patients diagnosed with 2nd line LPA.

A consequence of the implementation of direct testing on clinical samples opposed to culture isolates, i.e. the change to the diagnostic algorithm, is availability of results significantly earlier, which supports the reasons for changing the diagnostic algorithm. This means that patients can be initiated onto the appropriate anti-TB therapy in a much shorter time, and thus be contagious in the community for less time. The laboratory turnaround time for 1st and 2nd line LPA is still substantial and this delay could be consequent of the higher rates of inconclusive results in cohort 2, as a result of the change in the algorithm to include direct testing.

In the comparison of 1st and 2nd line LPA and phenotypic DST results, it was found that 1st line DST showed almost a perfect agreement with phenotypic DST for the detection of both RIF and INH, with no discordance found for RIF resistance, and two discordant results for INH resistance. Second line LPA showed moderate agreement with phenotypic DST for FLQ resistance, with only 4 discordant results. Furthermore, 2nd line LPA demonstrated a substantial agreement with phenotypic DST for INJ resistance, with only 3 discordant results. Thus, we can concur that 1st and 2nd line LPA is comparable to phenotypic DST for the detection of resistance, and justified it's use as a faster method for reflex testing for DR-TB.

Unfortunately, the improvement in TB diagnostics is not benefitting patients optimally. Although the speed of TB diagnostic tests has increased remarkably, there are still delays in getting the results from the laboratory to the health care facility, there are often delays in health care facilities contacting patients and it takes time for patients to get to the facility. There was an increase in the time taken between the availability of LPA result and treatment initiation in cohort 2. This is a consequence of the problem in getting patients to return to the facility for both further testing and treatment. In cohort 1, the algorithm dictated that patients were hospitalized during DR-TB care, thus explaining the reduced delay between the availability of results and treatment initiation. A number of socio-economic factors delay patients return to facilities, including limited transport options, lack of finances and shortage of leave from work. It has been shown that increased distance from patients' homes to health facilities is a significant predictor of delayed treatment initiation (75, 76). The study population is from a rural area, which would impact on how far patients have to travel to seek healthcare, family support and household responsibilities. (75, 76)

In a study by Jacobson et al, it was observed that the introduction of LPA did reduce the time to treatment initiation, but improvements in health system infrastructure were required in order to see the greatest impacts from new diagnostics (56). Although my study showed a reduction in time to

appropriate treatment initiation, the full impact of 2nd line LPA was probably not seen in the first year of implementation as the implementation of new diagnostics and new algorithms often take some time to become embedded into routine practice.

The treatment outcomes between the two cohorts did not significantly improve, with the successful outcome rate increasing from 61.0% in cohort 1, to 63.7% in the second (*p=0.763*). Although Padayatchi, *et al*. found that there was no significant improvement in treatment outcomes associated with reduced time to treatment initiation (77), we, however, did find a 50% improvement in treatment outcomes for XDR-TB patients in cohort 2. This improvement in treatment outcomes cannot be exclusively attributed to the reduction in time to treatment initiation, as changes to the health system, such as decentralisation and improvements in anti-TB drugs, may have contributed to these positive changes.

Furthermore, the results demonstrated that there was no significant difference in successful treatment outcomes in HIV positive patients between the two time periods (*p=0.763*). A study into the management of patients requiring both ART and TB drugs, however, suggested that there are interactions between antiretroviral and TB medications that compromise concomitant treatment implementation in limited resource settings (78), and thus further negating the positive effects on outcome of the improved algorithm. The most successful treatment outcomes occurred in patients that were initiated onto appropriate treatment within 6 – 15 days after sputum collection. This is ostensibly due to the fact that MDR/RR-TB makes up the greatest proportion of DR-TB in the both cohorts, diagnosed by Xpert, proven to have a rapid laboratory turnaround time, as a predictor of MDR-TB. Thus, patients are initiated onto treatment more quickly, and as the research has shown, this is associated with better treatment outcomes. This notion is further supported by evidence of the treatment changes. The treatment changes in cohort 1 were primarily based on diagnostic testing, suggesting that the initial treatment regimen was not appropriate. Cohort 2 saw fewer treatment changes and those patients that did have their regimen altered, were mostly due to adverse reactions to therapy. This indicates that in cohort 2, appropriate treatment was initiated in the first instance, and changes were largely made due to adverse reactions and not drug resistance development.

1st and 2nd line LPA proved comparable to phenotypic DST for resistance detection, thus reinforcing the decision to use this much faster method for MDR- and XDR-TB diagnosis. The problem, however, that arose with direct sputum testing was the increase in inconclusive results. The cause for the higher inconclusive results is potentially due to reduced bacillary load in the clinical samples, opposed to indirect 1st and 2nd LPA that uses culture isolates. These patients then have to wait for phenotypic DST results to become available before commencement of appropriate therapy, thus not taking advantage of the faster DST that 1st and 2nd line LPA provides. These results are comparable with literature, as a study performed in the Western Cape of South Africa to ascertain the value of direct LPA to predict 2-month positivity in liquid culture, found 26% of samples were inconclusive using direct LPA (79).

Additionally, an Ethiopian study found that of 274 presumptive MDR-TB patients, 30.8% reported inconclusive direct LPA results (80). This reinforces the need to improve direct testing LPA protocol.

8. Limitations

For this study, data were used from the routine databases, routinely collected by health workers, which were, at times, incomplete. In an operational setting, missing data was a challenge, specifically missing laboratory results which with the sample size of the two time periods, could possibly skew data. It is unclear if these data were missing or whether the diagnostic tests were not carried out. There were patient records that did not have Xpert results recorded on the EDR, but only LPA. This reduced the number of patients who could be included in the statistical analysis, causing possible bias. In addition to missing data, the routine data was at times inaccurately captured. This is a limitation often reported in retrospective studies using routine data. Additionally, the small cohort sizes were small and limited the analyses that could be done. Further studies including more facilities should be performed to achieve this objective. In the Western Cape databases have been linked so that results from the NHLS LIMS system are directly imported into the EDR. This would negate human error and ensue the EDR is updated with laboratory results timeously. At this time, there is no linkage between the two databases in the Umzinyathi district, but this should be a future aim.

Only patients who were initiated onto treatment were enrolled in the EDR, thus the patients that were diagnosed with DR-TB but never treated remain unaccounted for in this study. Further investigation into the follow-up of these patients should be done.

The district researched for this study was purposively selected on the basis of their good TB programme implementation and monitoring, consequently this district may not be representative of other districts in South Africa. Furthermore, the Umzinyathi district of KwaZulu-Natal is a rural district, thus further studies to include urban districts needs to be done in order to validate the results.

To reduce the time taken for the laboratory to send the results to the public health facility, as well as the time taken to contact patients to return to the facility for treatment, an SMS flagging system or smartphone application, could be put in place. Thus, the health facility and the patient are simultaneously informed that results are available, immediately after the laboratory has released them.

Furthermore, mobile clinics can be implemented to distribute TB-management at community level to remote areas. This will not only aid active case finding, but ensure samples are collected more timeously and patients initiated onto treatment as quickly as possible.

9. Conclusion

This study has demonstrated that the introduction of direct 1st and 2nd line reflex testing in the diagnostic algorithm has reduced the laboratory turnaround time for DR-TB. However, the challenges in health

systems and processes continue to delay patient initiation on therapy, thus negating the advantages of the improved diagnostics.

In a rural district in KwaZulu-Natal, I have shown that time to appropriate treatment initiation for patients with XDR-TB was reduced by the introduction of the 2nd line LPA, and as a consequence there were more successful treatment outcomes in these patients. However, further studies are needed to see if these results are generalisable to other areas in KwaZulu-Natal and South Africa.

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10. APPENDICES

Appendix A – Tables

A1: Number of patients initiated on treatment based on Xpert, LPA, DST or clinical diagnoses

	2015/2016 N=141	2017/2018 N=102	P-value
	N (%)	N (%)	
Placed on treatment based on Xpert	113 (80.1%)	73 (71.6%)	0.832
Placed on treatment based on LPA	0 (0.0%)	11 (10.8%)	
Placed on treatment based on Phenotypic DST	17 (12.1%)	12 (11.8%)	0.679
Placed on treatment based on Clinical Indication	11 (7.8%)	6 (5.9%)	0.466

A2: Binary logistic regression to illustrate the predictors of successful treatment outcomes

	Estimate	p-value	OR	95% C.I.for EXP(B)	
				Lower	Upper
Age at Treatment Start	-.027	.067	.974	.946	1.002
Gender	-.458	.163	.633	.333	1.204
INH mono	1.234	.453	3.436	.137	86.328
MDR-TB	1.644	.011	5.174	1.463	18.296
PreXDR-TB	1.136	.304	3.113	.357	27.123
RR-TB	.862	.172	2.367	.688	8.147
PT1	.345	.754	1.412	.163	12.203
PT2	.099	.929	1.104	.127	9.599
PTB	.411	.633	1.509	.279	8.168
HIV negative	.248	.608	1.281	.497	3.301

	Estimate	p-value	OR	95% C.I.for EXP(B)	
				Lower	Upper
Not on Short Regimen	1.017	.035	2.764	1.075	7.106
Days to Appropriate Treatment Initiation	0.009	.196	1.009	.996	1.022
Not on BDQ	-1.146	.014	.318	.128	.790

A3: Comparison of Xpert rifampicin resistance results with phenotypic DST

			1st line LPA RR Result		Total
			Sensitive	Resistant	
Xpert RR Result	Sensitive	N (% within LPA RR Result)	0 (0.0%)	1 (1.5%)	1 (0.4%)
	Resistant	N (% within LPA RR Result)	7 (100%)	64 (98.5%)	71 (98.6%)
Total		N (% within LPA RR Result)	7 (100.0%)	65 (100%)	72 (100%)

A4: Comparison of Xpert rifampicin resistance results with 1st line LPA rifampicin resistance

			DST RR Result		Total
			Sensitive	Resistant	
1st line LPA RR Result	Sensitive	N (% within DST RR Result)	1 (100.0%)	0 (0.0%)	1 (1.2%)
	Resistant	N (% within DST RR Result)	0 (0.0%)	84 (100.0%)	84 (98.8%)
Total		N (% within DST RR Result)	1 (100.0%)	84 (100.0%)	85 (100.0%)

A5: Comparison of 1st line LPA rifampicin resistance results with phenotypic DST results

			DST INH		Total
			Sensitive	Resistant	
1st line LPA INH-R Result	Sensitive	N (% within DST INH Result)	28 (93.3%)	1 (1.9%)	29 (34.5%)
	Resistant	N (% within DST INH Result)	2 (6.7%)	53 (98.1%)	55 (65.5%)

		DST INH		Total
		Sensitive	Resistant	
Total	N (% within DST INH Result)	30 (100.0%)	54 (100.0%)	84 (100.0%)

A6: Comparison of 1st line LPA isoniazid resistance results with phenotypic DST results

			DST FLQ-R		Total
			Sensitive	Resistant	
2nd line LPA FLQ-R	Sensitive	N (% within DST FLQ-R)	36 (64.3%)	0 (0.0%)	36 (61.0%)
	Resistant	N (% within DST FLQ-R)	4 (7.1%)	3 (100.0%)	7 (11.9%)
	Uninterpretable	N (% within DST FLQ-R)	16 (28.6%)	0 (0.0%)	16 (27.1%)
Total		N (% within DST FLQ-R)	56 (100.0%)	3 (100.0%)	59 (100.0%)

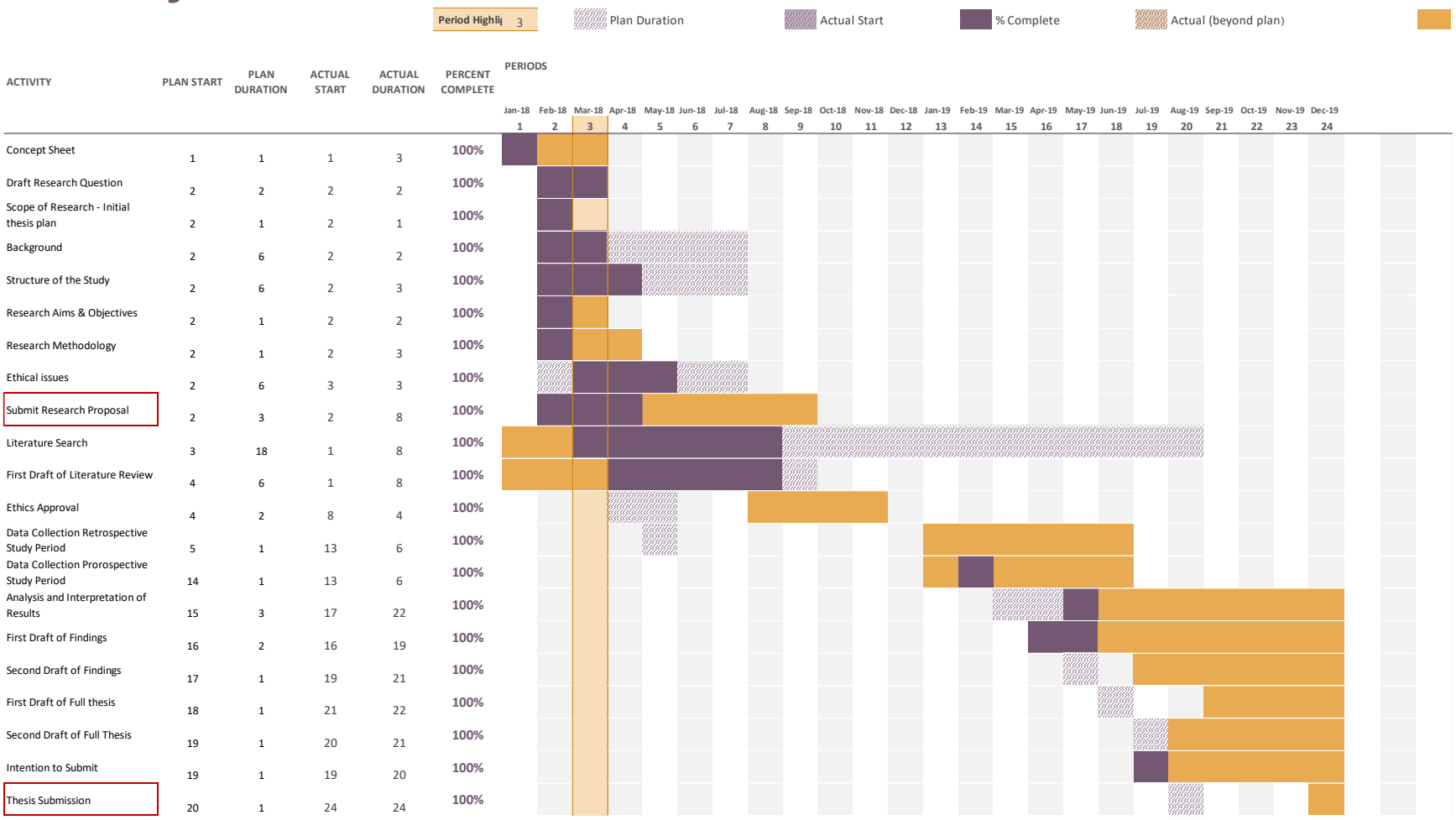
A7: Comparison of 2nd line LPA fluoroquinolone resistance results with phenotypic DST results

			DST INJ-R		Total
			Sensitive	Resistant	
2nd line LPA INJ-R	Sensitive	N (% within DST INJ-R)	34 (64.2%)	0 (0.0%)	34 (58.6%)
	Resistant	N (% within DST INJ-R)	3 (5.7%)	5 (100.0%)	8 (13.8%)
	Uninterpretable	N (% within DST INJ-R)	16 (30.2%)	0 (0.0%)	16 (27.6%)
Total		N (% within DST INJ-R)	53 (100.0%)	5 (100.0%)	58 (100.0%)

A8: Comparison of 2nd line LPA injectables resistance results with phenotypic DST results

Appendix B – Gantt Chart

MSc Project Planner



ACTIVITY	PLAN START	PLAN DURATION	ACTUAL START	ACTUAL DURATION	PERCENT COMPLETE
Concept Sheet	1	1	1	3	100%
Draft Research Question	2	2	2	2	100%
Scope of Research - Initial thesis plan	2	1	2	1	100%
Background	2	6	2	2	100%
Structure of the Study	2	6	2	3	100%
Research Aims & Objectives	2	1	2	2	100%
Research Methodology	2	1	2	3	100%
Ethical issues	2	6	3	3	100%
Submit Research Proposal	2	3	2	8	100%
Literature Search	3	18	1	8	100%
First Draft of Literature Review	4	6	1	8	100%
Ethics Approval	4	2	8	4	100%
Data Collection Retrospective Study Period	5	1	13	6	100%
Data Collection Prorospective Study Period	14	1	13	6	100%
Analysis and Interpretation of Results	15	3	17	22	100%
First Draft of Findings	16	2	16	19	100%
Second Draft of Findings	17	1	19	21	100%
First Draft of Full thesis	18	1	21	22	100%
Second Draft of Full Thesis	19	1	20	21	100%
Intention to Submit	19	1	19	20	100%
Thesis Submission	20	1	24	24	100%

Appendix C – GCP Certificates

Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Tamara Sneyd** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 03/09/2018.

Certification Number: 2669291.

A.1 Certificate of successful completion of NIH GCP course

TRREE

Zertifikat
Certificat
Certificado
Certificate

Promouvoir les plus hauts standards éthiques dans la protection des participants à la recherche biomédicale
Promoting the highest ethical standards in the protection of biomedical research participants

Clinical Trials Centre
The University of Hong Kong

Certificat de formation - Training Certificate

Ce document atteste que - this document certifies that

Tamara Sneyd

a complété avec succès - has successfully completed

Introduction to Research Ethics

du programme de formation TRREE en évaluation éthique de la recherche
of the TRREE training programme in research ethics evaluation

September 26, 2018
CID : 5V4ookO6BHI

Professeur Dominique Sprumont
Coordinateur TRREE Coordinator

FMH Continuing Education Program (5 Credits) Programme de Formation continue (5 Crédits)

Foederatio Pharmaceutica Helvetiae FPH Programmes de formation continue Continuing Education Programs Programmes de formation continue

Ce programme est soutenu par - This program is supported by :
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[REV : 20170310]

A.2. Certificate of Successful Completeion TREE Introduction to Research Ethics

Appendix D – Brief Curriculum Vitae of principal investigator

Tamara Sneyd
Bracken Farm, Greytown, 3250 | 084-2082120 | tamarasneyd@gmail.com

Education

BACHELOR OF SCIENCE | 2002 | UNIVERSITY OF KWAZULU-NATAL

- Major: Biomedical Science
- Related coursework: Genetics and Evolution, Diversity of Life, Chemistry 1A1, Physics in the Life Sciences, From Molecules to Organisms, Processes and Structures of Life, Chemistry 1A2, Human Body: Form and Function, Biochemistry, Basic Immunology, Environmental Microbiology, Cardiovascular/ Respiratory Physiology, Neurophysiology, Protein Structure and Function, Molecular biology, Microbiology and Health, Endocrine and Renal Physiology, Gastrointestinal Physiology and Blood, Functional Cell Architecture, Comparative Immunology, Environmental Toxicology, Medical Bio-statistics, Research Project – Virology, Molecular Virology, Bioethics, Principles of Biotechnology, Medical Microbiology, Bioenergetics, Neuro-endocrinology, Wound Healing
- Dean's Commendation: 2002, 2003, 2004
- Dean's List of High Achievers: 2002, 2003, 2004
- Certificate of Merit in Neuro-endocrinology (First in Class)
- Graduated with Distinction in Major Subjects

BACHELOR OF SCIENCE HONOURS | 2005 | UNIVERSITY OF PRETORIA

- Major: Medical Criminalistics
- Related coursework: Forensic Medicine (Pathology), Ballistics, Anthropology, Odontology, Criminalistics, Toxicology, Applied Research Methodology, Biostatistics, Medical Law, Practical Component: Medico-legal autopsies, Crime scene attendance, Court case attendance, Laboratory skills, Research protocol
- Graduated with Distinction

Experience

BUSINESS OWNER | PEN & INK | MAY 2017 - PRESENT

- Editing and writing business specializing in academic writing, journal articles, as well as news, features and copywriting.

ADMINISTRATOR | UMVOTI TYRES & BATTERIES | JUL 2016 – MAY 2017

- Bookkeeping and administrative functions

SHERQ ADMINISTRATOR | MASONITE | NOV 2013 – JUN 2016

- Implementation and maintenance of the risk management software, Auditing, Document control and curatorship.

NURSERY MANAGER | SUTHERLAND SEEDLINGS | JUNE 2009 – DEC 2012

- Planting Operations, Establishment and administration of ISO 9001:2008 Quality Management System
- Research, Nursery Statistics, Establishment of Forestry Clonal and Pine hybrid operations, Customer follow up service and Sales Repping, Advertisements

DAIRY MANAGER | OLIVAR FARM | JUL 2008 – MAY 2009

- Herd fertility – Oestrus synchronisation and AI, Herd health and maintenance, Basic Veterinary Procedures, Milk production and recording, Calf rearing, Heifer management, Pasture management, Feed utilisation and management, Vaccination programmes

ENVIRONMENTAL OFFICER | SAPPI SAICCOR | MAY 2007 – JUN 2008

- Ambient emissions monitoring and reporting, Air dispersion modelling, Plant optimisation

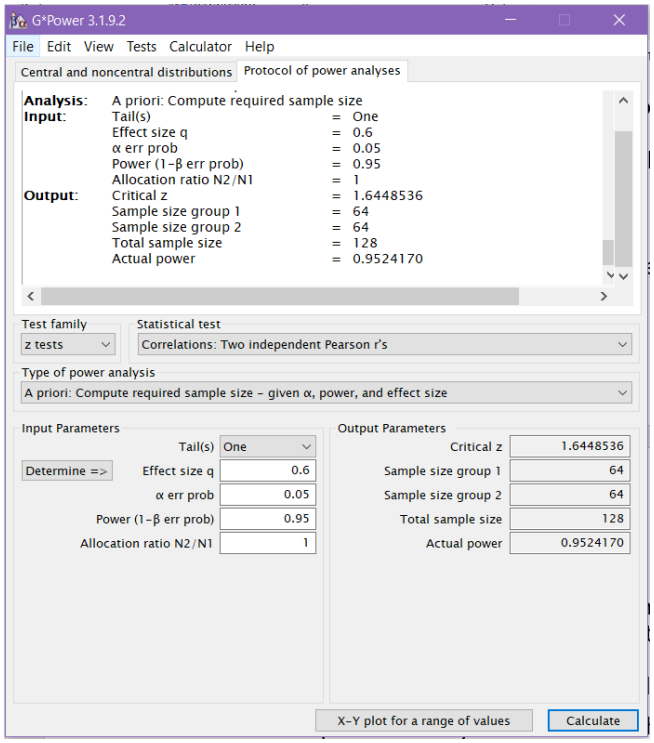
SHEQ SUPERINTENDENT | SAPPI SAICCOR | APR 2008 – JUN 2008

- Uphold SHEQ policies and procedures within the factory including: Fire escape systems, safety installations, evacuation programs, hazard identification and risk assessments, non-conformance reporting and investigation, root cause analysis and incident investigation, adherence to environmental objectives and targets, training issues, ISO documentation and auditing.

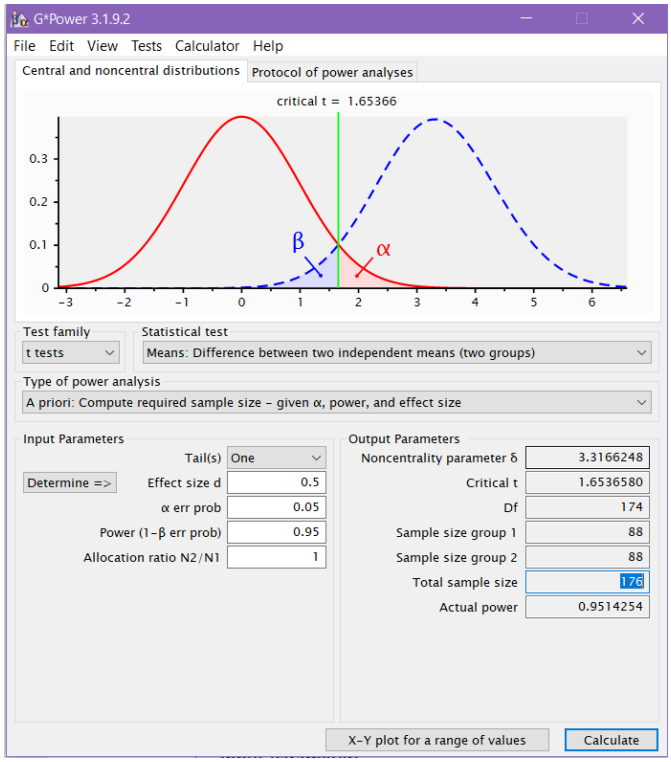
CHEMICAL TECHNOLOGIST | SAPPI SAICCOR | APR 2006 – MAY 2007

- Investigation and optimization of the chemical analyses and processes, as well as research and trials.

Appendix E – G-power tests for determination of statistical feasibility of sample size



Sample size for Pearson's correlation



G*Power calculation to determine adequate sample size for the dataset at 95% confidence interval and 0.05 α.

Proposed statistical information for comparison of the two groups:

t tests - Means: Difference between two independent means (two groups)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	One
	Effect size d	=	0.5
	α err prob	=	0.05
	Power (1-β err prob)	=	0.95
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	3.3166248
	Critical t	=	1.6536580
	Df	=	174
	Sample size group 1	=	88
	Sample size group 2	=	88
	Total sample size	=	176
	Actual power	=	0.9514254

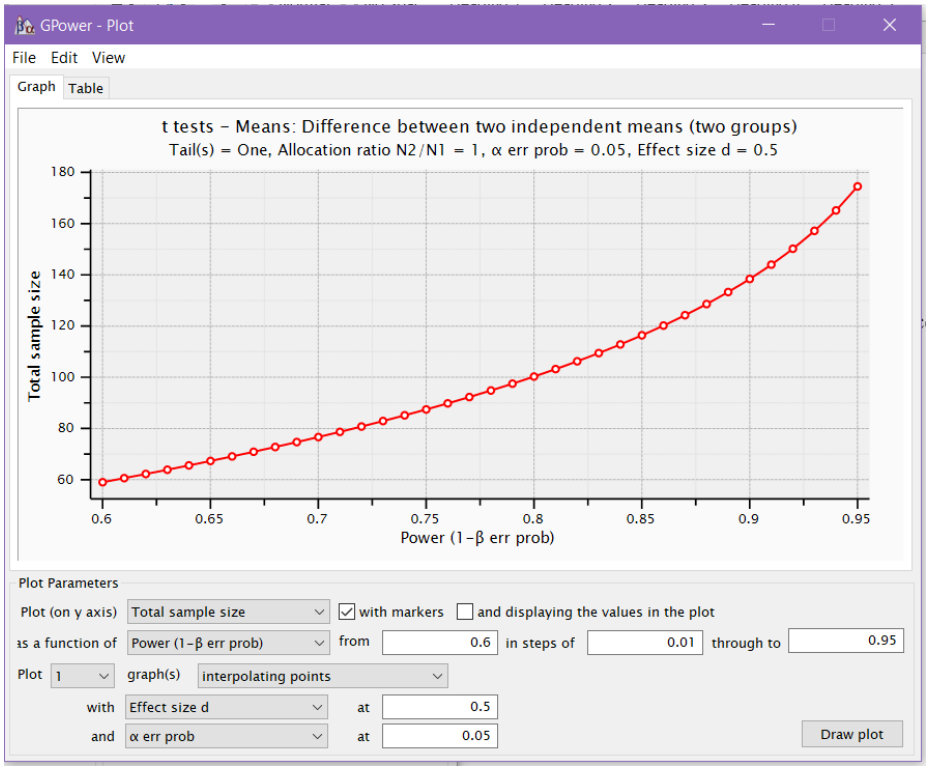


Fig D.3. X/Y plot demonstrating the statistical power relative to sample size

Appendix F – Budget

Budget Item	Comment	Reason for Expense	Monthly	1 year	2 years
Running Expenses					
Consumables					
Internet subscription	Cell C internet subscription R788/m. Half will be for Masters work	Online research and communications with Supervisors	R 394,00	R 4 728,00	R 9 456,00
Paper	Ream of paper R56/ream and average 1 ream per month	Printing of journal articles, research materials and	R 56,00	R 672,00	R 1 344,00
Pens	R12 per pen and average 2 per month		R 24,00	R 288,00	R 576,00
Staples	R15 per box and average 5 per year		R 15,00	R 75,00	R 150,00
Files	R60 and require 2 per year		R 60,00	R 120,00	R 240,00
Post It Notes	R80.50 per cube and require 4 per year		R 80,50	R 322,00	R 644,00
File Dividers	R10.00 each and require for 4 files		R 10,00	R 20,00	R 40,00
Highlighters	R79.80 per pack, will need 2 per year	Stationary consumables	R 79,80	R 159,60	R 319,20
Small Items of Equipment	1TB Portable Hard drive	Back up of research	R 829,00	R 829,00	R 829,00
	Canon printer, scanner and fax machine	Printing and scanning as I live in a rural area	R 499,00	R 499,00	R 499,00
Travel	Travel Expenses	Once a month supervisor meetings 300km (Greytown to Durban) at AA rate of R6.66/km	R 1 998,00	R 23 976,00	R 47 952,00
Cost of Photocopying/Printing	Canon 445 and 446 printer cartridges cost R279 and R289 respectively replace every 2 months.	Printing consumables	R 568,00	R 3 408,00	R 6 816,00
Total Costs:			**R 4 613,30**	**R 35 096,60**	**R 68 865,20**

Proposed budgetary requirements for the study

Appendix G – BREC Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

10 December 2018

Ms T Sneyd (202500797)
School of Laboratory Medicine and Medical Sciences
College of Health Sciences
tamarasneyd@gmail.com

Protocol: The impact of the introduction of direct first and second-line reflex testing in the management of drug resistant Tuberculosis at Greytown Hospital, UMzinyathi district, KwaZulu-Natal.
Degree: MSc **BREC REF: BE635/18**

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received 15 October 2018.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 29 November 2018 to BREC correspondence dated 31 October 2018 has been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval** and may begin as from 10 December 2018. Please ensure that site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from 10 December **2018.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **noted** by a full Committee at its next meeting taking place on **11 December 2018.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor V Rambiritch
Chair: Biomedical Research Ethics Committee

Supervisor: Prof K Mlisana co-Supervisor: Dr M Loveday Postgrad admin: dudhraj@ukzn.ac.za

Appendix H – NHLS Approval

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6142
Fax: +27 (0)11 386 6296
Email: babatyi.kgokong@nhls.ac.za
Web: www.nhls.ac.za

11 March 2019

Applicant: Mrs Tamara Sneyd
Institution: University of Kwazulu-Natal
Department: School of Laboratory Medicine and Medical Science
Email: tamarasneyd@gmail.com
Cell: 084 208 2120

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"The impact of the introduction of direct first and second-line reflex testing in the management of drug-resistant Tuberculosis at Greytown Hospital, Umzinyathi district, Kwazulu-Natal"** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application.

Please note that final approval will be granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Ethics approval is obtained from a recognised SA Health Research Ethics Committee.
- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.

Please note that this letter constitutes provisional approval by the NHLS Academic Affairs and Research Office. Once Ethics approval has been granted please send it to academic.research@nhls.ac.za. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix I – Department of Health Approval

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Physical Address: 330 Langalibalele Street, Pietermaritzburg
Postal Address: Private Bag X9051
Tel: 033 395 2805/ 3189/ 3123 Fax: 033 394 3782
Email:
www.kznhealth.gov.za

DIRECTORATE:
Health Research & Knowledge Management

Ref: KZ_201811_005

Dear Mrs T Sneyd
(UKZN)

Subject: Approval of a Research Proposal:

1. The research proposal titled '**The impact of the introduction of direct first and second-line reflex testing in the management of drug-resistant Tuberculosis at Greytown Hospital, Umzinyathi district, KwaZulu-Natal**' was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at Greytown Hospital.

2. You are requested to take note of the following:

   a. *Kindly liaise with the facility manager BEFORE your research begins in order to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.*
   b. *Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.*
   c. *Provide an interim progress report and final report (electronic and hard copies) when your research is complete.*

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 3/11/18

Appendix J – Permission from Greytown TB Hospital

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE MEDICAL MANAGER
GREYTOWN PROVINCIAL HOSPITAL
Private Bag X 5562, Greytown, 3250
Bell street, Greytown, 3250
Tel.: 033 413 9400 Fax: 033 413 2809
Email:Morgan.Govender3@kznhealth.gov.za
www.kznhealth.gov.za

21 November ,2018
Enquiries: DR M A Govender

To:
The Principal/Protocol Investigators,
Ms Tamara Sneyd

Title: The impact of the introduction of direct first and second-line reflex testing in the management of drug-resistant Tuberculosis at Greytown Hospital, Umzinyathi district, Kwazulu-Natal

I have pleasure in informing you that permission has been granted to you for conducting research as stipulated above.
Please note the following:

1. Please ensure that you adhere to all the policies, procedures, protocols and guidelines of the Department of Health with regards to this research.
2. This research will only commence once this office has received approval of your study from the Provincial Health Research and Ethics Committee (PHREC) in the KZN Department of Health..
3. Please ensure this office is informed before you commence your research.
4. Greytown Provincial Hospital will not provide any resources for this research.
5. You will be expected to provide feedback on your findings to this office

Thanking You,

Yours sincerely,

DR Morgan Govender
MEDICAL MANAGER

Signature .....