GENEXPERT DATA FOR RIFAMPICIN RESISTANCE IN SWAZILAND – A RETROSPECTIVE ANALYSIS FROM 2012–2016

by

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DECLARATION

I declare that **GENEXPERT DATA FOR RIFAMPICIN RESISTANCE IN SWAZILAND – A RETROSPECTIVE ANALYSIS FROM 2012–2016** is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

I further declare that I submitted the dissertation to originality checking software and that it falls within the accepted requirements for originality.

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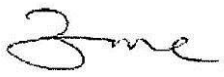

02 July 2019

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ABSTRACT

Surveillance of anti-TB drug resistance is key for sustained TB control. GeneXpert may provide a new avenue at least for Rifampicin resistance surveillance. This study used GeneXpert data to determine epidemiological prevalence of rifampicin resistance in Swaziland from 2012–2016.

Nine laboratories in Swaziland were purposively selected and from them 50% of GeneXpert records were included in the study. Data was analysed using SAS studio version 9.04.

Of the 5418 GeneXpert records included, 50.8% were from males. The prevalence of rifampicin resistance was 10.5%, 10.8%, 11.3, 11,3 and 11,8 for 2012, 2013, 2014, 2015 and 2016 respectively. Further, probe E was the most common locus of mutation conferring rifampicin resistance accounting for 62.2% of all resistance.

This study showed that routine data from GeneXpert can be used for epidemiological surveillance of rifampicin resistance and can be used to develop a simple continuous system of rifampicin resistance surveillance.

Keywords

Descriptive correlational; GeneXpert; new TB patients, previously treated TB patients; Rifampicin resistance; anti-tuberculosis drug resistance surveillance; molecular probes; Xpert MTB rif, rpoB gene; rifampicin resistance patterns; rifampicin resistance proportion.

KUSHANDISWA KWERUZIVO RUNOBVA PAGENEXPERT KUTSANANGUDZA HUKUTU HWERURINDI KUMUSHONGA WERIFAMPICIN MUSWAZILAND MUMAKORE A2012 KUSVIKA 2016

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Pfupiso

Cherechedzo yehukukutu hwemabhakitiriya anokonzera rurindi, kumishonga kunobatsira kurwisa kwechirwere cherurindi. Kubva pasichigare, ruzivo rwemamiriro ehukukutu hwemabhakitiriya anokonzera rurindi rwaiwanikwa kubva kuwongororo yekukudza mabhakitiriya muchikafu chinemishonga. Inodaidzwa kuti culture and DST muchirungu. Saka, tsvagurudzo ino iri maererana nekutsvaga huwandu hwehukukutu hwerurindi kumushonga werifampicin munyika yeSwaziland semawanikirwe azvakaitwa neGeneXpert mumakore anoti 2012 kusvikira 2016.

Pakutanga zvipatara zvipfumbamwe zvakasarudzwa mavune kuti varwere vachapinda musarudzo vange vachibva kwazviri. Mudanho repiri chikamu chimwe muzviviri zvevarwere vese vakanga vabatwa rurindi neGeneXpert mumakore 2012–2016 vakasarudzwa kuti vapinde mutsvagurudzo. Ruzivo pamusoro pevakasarudzwa rwakaunganizdwa, nokukwenzverwa ruchibva rwaongororwa neSAS version 9.04

Varwere 5418 ndivo vakapinda muongororo zvikawanikwa kuti chikamu chinoit 50.8 kubva muzana vakanga vari vechirume. Zvakare mumakore anoti 2012 zvichienda kuna 2016 varwere vakanga vaine rurindi rwune hukukutu kumushonga werifampicin vaiva zvikamu zvinoti 10.5%, 10.8%, 11.3, 11,3 ne 11,8 kubva muzana zvichiendera nemakore. Uyezve, rupandi rwechishanu (probe E), ndirwo rwakanga ruri rwakajairika senzvimbo inobva hukukutu hwerurindi kumushonga werifampicin. Probe E yaisanganisira zvikamu 62.2 kubva muzana zvenzvimbo dzese dzaibva hukukutu. Yaiteverwa ne Probe D, B, C na A idzo dzaisanganisira zvikamu 25.3, 8.6, 2.5 ne 1.3 kubva muzana zvichienda zvakadaro.

Tsvakurudzo ino yakaratidza pachena kuti GeneXpert inogona kushandiswa kuunganidza ruzivo rwehukukutu hwerurindi kumushonga werifampicin. Uyezve GeneXpert inogona kutaridza mafambire anoita hukukutu hwerurindi kumushonga

werifampicin nekufamba kwenguva, zvikamu zvevanhu nekwavanogara. Saka munyika dzisina kunyanyobudirira, ruzivo runobva kuGeneXpert runogona kushandiswa zviri nyore kugadzira cherechedzo yehukukutu hwerifampicin.

SISINDO SEKUGWAMA KWALELI GCIWANE EMUTSINI WE IRIFAMBICIN NGEKUHLOLA KWEGENEXPERT ESWATINI KUSUKELA NGEMNYAKA WA2012 KUYA KU2016

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PUNGULO

Lucwaningo lwekuhlola kugwama kweligciwane lesifuba sengati (TB) kumitsi lubalulekile kulamwula sifo sesifuba sengati eveni. Lolucwaningo lutoveta bukhulu benkinga leyentiwa ngulokugwama nekuveta imiphumela yetindlela letikhona tekulapha nekufundzisa ngalo leligciwane. Indlela lebeyivamile yekupopola kugwama kweligciwane bekungu Phenotypic DST, kodwa bakaMhlaba Betemphilo sebapasise tindlendlela letinsha (Molecular methods) letifaka ekhatsi iGeneXpert. Lolucwaningo luveta sisindo sekugwama kwaleli gciwane emutsini we iRifambicin ngekuhlola kweGeneXpert Eswatini kusukela ngemnyaka wa2012 kuya ku2016.

Lolucwaningo lwaba tigaba letimbili tekukhetsa tindzawo tekulwenta. Sigaba sekucala safaka emaLaboratory lasishiya galolunye (9) kuletifundza letine telive. Sigaba sesibili saba ngalokunge mashumi lasihlanu ekhulwini (50%) wawo wonke imphumela yeGeneXpert. Letinombolo letatfolakala lapho tahlungwa ngeSAS Studio 9.04.

Tinkhulungwane letisihlanu emakhulu lamane lanelishumi nesiphohlongo (5418) bangelela lolucwaningo, kubo labangemashumi lasihlanu nencenye bebadvuna. Imphumela yekugwama kwemutsi weRifambicin beyime kanje 10.5%, 10.8%, 11,3% 11.3%, na11.8% ngeminyaka lelandzelana kanje 2012, 2013, 2014, 2015 na2016. Phinze kwavela kutsi Probe E nguye abenetibalo letisettulu kuRifambicin ngalokungemashumi lasitfupha ekhulwini nencenye (62.2%). Kululokunye kwabonakala kutsi Proe D, B, C naA ngekulandzelana baba ngu 25.3%, 8.6%, 2.5% na1.3% alokugwama kweligciwane.

Lolucwaningo lwakhomba kutsi tinombolo tekupopola ngeGeneXpert tingakhomba kutsi babani labanenkinga lenkulu yekugwama phindze tikhombe kutsi bebabangaki futsi babulili buni bebanaleligwamile ngetikhatsi letehlukene netindzawo. Lolucwaningo luvete kutsi emave lanemnotfo nontengantengako angasebentisa lucwaningo loluchubekako

lolukhomba linani lekugwama kuRifambicin kwaleligciwane kusetjentiswa tinombolo tekupopola teGeneXpert.

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My supervisor Dr MG Makua who guided me from the beginning to the end. To her I say thanks for being there all the time.

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Lastly, I thank God who created and allows us to create.

Dedication

To my two daughters Eleanor and Zohar, for they were untiring and unfailing in their efforts to keep me hydrated while I was writing.

And to my twitter followers who I partially abandoned during the process of writing, sorry mates but this had to be done.

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LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
AII	Airborne Infections Isolation
BCG	Bacille Calmette Guerin
BSL	Biosafety Level
CI	Confidence Interval
CT	Cycle threshold
DNA	Deoxyribonucleic Acid
DST	Drug Sensitivity Testing
FDA	Federal Drug Administration
FIND	Foundation for Innovative New Diagnostics
GLI	Global Laboratory Initiative
H	High
HBC	High Burden Country
HBDC	High Burden Developing Country
HIV	Human Immune deficiency Virus
HSREC	Health Studies Research Ethics Committee
L	Low
LMIC	Low to Medium Income Countries
M	medium
MDR	Multidrug resistant TB
Ms Excel	Microsoft Excel
MSF	Medecins Sans Frontieres
MTB	Mycobacterium Tuberculosis
NGO	Non-Governmental Organization
NHRRB	National Health Research Review Board
NPV	Negative Predictive Value
NTCP	National Tuberculosis Control Program
PCR	Polymerase Chain Reaction
Rif	Rifampicin
RNA	Ribonucleic Acid

RRDR	Rifampicin Resistance Determining Region
SHLS	Swaziland Health Laboratory Services
SPC	Sample Processing Control
TB	Tuberculosis
UN	United Nations
UNISA	University of South Africa
USC	University of Southern California
VL	Very low
WHO	World Health Organization
XDR	Extremely Drug Resistant TB
Xpert	GeneXpert

CHAPTER 1

ORIENTATION TO THE STUDY

1.1 INTRODUCTION

This introductory chapter sets the background for the reader to understand the advent and the subsequent uses of the GeneXpert and its data for simultaneous detection of TB and rifampicin resistance. The chapter also seeks to orient the reader with the major directions of the study thus, in here is also stated the problem statement, the aims of the study, its significance and the study design. At the end of the chapter there is a brief description of what to expect in each of the remaining chapters.

1.2 BACKGROUND TO THE PROBLEM

Over the past few decades antimicrobial resistance has emerged as an important global public health problem. In the case of Tuberculosis (TB), the emergence of resistance to anti-tuberculosis drugs threatens to roll back progress made in tuberculosis control (WHO 2017a:3, 2018a:76). According to Millard, Ugarte-Gil and Moore (2015), drug resistant TB is relatively more difficult to detect and treat successfully as evidenced by the requirement for relatively sophisticated diagnostic methods for detection and a low overall treatment success rate of about 50%. For a very long time the most widely available method of TB detection was smear microscopy. Its weaknesses include, limited sensitivity and inability to detect the resistance profile (Caulfield & Wengenack 2016:[34], WHO 2015a:14). Thus, patients diagnosed as having TB by smear microscopy, would need a further TB culture examination in order to be able to determine the resistance profile. However, culture and drug sensitivity testing are technically complex procedures which were and are still not widely available especially for Low and Middle-Income Countries (LMIC) (Millard et al 2015).

In the absence of alternatives, culture and Drug Sensitivity Testing (DST), have traditionally been relied on for providing data for clinical management of patients and surveillance of anti-TB drug resistance (WHO 2018b:2, 3). However, the reliance on culture and DST is problematic for TB control in LMIC in several ways. As pointed out by

Parsons, Somoskovi, Gutierrez, Lee, Paramasivan, Abimiku, Spector, Roscigno and Nkengasong (2011:[317–318]), there are often few laboratories capable of culture and DST in resource limited settings. Further, even when available, central TB laboratories in these settings also tend to be poorly equipped both in terms of biosafety and testing equipment. Joloba, Iragena and Onyebujoh (2013) state that a 2010 assessment of TB laboratories in Africa, showed that there were few labs that were capable of TB culture, let alone DST. Specifically, they found that in Africa there was on average one TB culture laboratory per 7.2 million population. Previously, it was considered ideal that there be on average one TB culture laboratory per 5 million population (WHO 2016a:2). Thus, in general, in Africa and other LMIC there is limited access to culture and DST.

To rationalize available culture and DST capacity, national TB control programs often implement algorithms that attempt to prioritize certain classes of patients for culture and DST albeit with negative consequences for clinical management of TB. For example, the diagnostic algorithm for Swaziland in 2008, prioritized culture and DST for patients at high risk of carrying drug resistant TB strains. These included contacts of drug resistant TB patients and patients who had failed to convert at two or three months. In that setting, while contacts of multidrug resistant (MDR), TB patients did not experience a delay in access to culture and DST, the patients failing to convert at two or three months of first line TB treatment, experienced delays in access to services (GKoS 2008:14). The apparent delay in diagnosis described here meant that some patients possibly continued to spread potentially drug resistant TB to their close contacts during the two or three months in which they were on ineffective treatment.

Moreover, limited access to DST also affects surveillance of anti-TB drug resistance. This is because logically it should follow that if a country is unable to provide culture and DST for clinical patient management then, it is also unable to provide the same for surveillance. This an argument may be supported by the findings of Cohen, Jenkins, Lu, McLaughlin, Floyd and Zignol (2014:[7]) that by 2014, Botswana was the only African country that had provided data for more than two drug resistance surveys since the beginning of the Global project on Anti-Tuberculosis drug resistance in 1994.

1.2.1 GeneXpert a possible gamechanger

The advent in late 2010, of GeneXpert, a WHO recommended rapid molecular test which simultaneously detects TB and rifampicin resistance in patient samples in late 2010 can be thought of as a turning point for rapid detection of TB as well as rifampicin resistance. Some authors have gone so far as suggesting that the GeneXpert should be called a 'game changer'. Cepheid ([s.a.]a), the manufacturer of the GeneXpert instrument, in the brochure of the second version of GeneXpert, use the language of 'game changer' in reference to GeneXpert. The headline of their brochure for the second version of GeneXpert instruments was the sentence:

> "we can't think of better words than game changing" (Cepheid [s.a.]a).

Undoubtedly GeneXpert is a game changer because it detects MTB and rifampicin resistance simultaneously with high sensitivity and specificity in two hours as opposed to at least three weeks needed for the combination of microscopy, culture and DST (MSF 2017). It is also a game changer because it was rapidly taken up by WHO member countries including ones where access to culture was previously limited. This rapid and widespread uptake of GeneXpert was also promoted by Cepheid's High Burden Developing Country (HBDC) program. Under the HBDC program 145 LMIC and NGOs working in them could purchase GeneXpert machines, cartridges and accessories at concessional prices (Cepheid [s.a.]b). Subsequently WHO (2016b) reports that by 2016, 130 of the eligible LMIC had procured GeneXpert.

In the case of Swaziland, GeneXpert was first implemented in 5 laboratories in mid 2011. Implementation was rapidly scaled up so that by 2012 there were 18 GeneXpert machines and by 2017 there were 68. Thus, in keeping with uptake elsewhere, GeneXpert also managed to be widely distributed in Swaziland fairly rapidly.

Of interest to this study was that the widespread distribution of GeneXpert also means widespread availability of GeneXpert data on rifampicin resistance and rifampicin resistance conferring mutations. Thus, from this study's viewpoint GeneXpert is possibly also a game changer at least for rifampicin resistance surveillance.

1.2.2 GeneXpert vs smear microscopy

Compared to microscopy, the GeneXpert provides a simple and rapid way of identifying, from the primary specimen, the presence of a strain belonging to the mycobacterium tuberculosis (MTB), complex in a patient specimen plus rifampicin resistance. Velayati and Farnia (2017:13) remark that the MTB complex is made up of 8 genetically homogenous species of mycobacteria that cause respiratory disease in humans. They are Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium microti, Mycobacterium canettii, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium suricattae, Mycobacterium mungi, Mycobacterium dassie, and Mycobacterium oryx.

Microscopy on the other hand can only go as far as identifying the presence of mycobacteria without identifying whether the infective agent belongs to mycobacterium tuberculosis complex or not (Steingart, Schiller, Horne, Pai, Boehme & Dendukuri 2014:[4]). The obvious disadvantage is not only that for clinical management it is at least important to identify if the infective species is from the MTB complex (as the majority of Tuberculosis in human is caused by MTB complex species), but also that technical errors such as using unfiltered tap water in the laboratory may also lead to false positives on microscopy. Environmental mycobacteria may easily be found in unfiltered tap water thus easily deposited on microscopy slides during washing steps (Tu, Chen, Huang, Huang, Chen, Liu, and Lin 2007:[6296]). Further, from a clinical management point of view, infection with non-tuberculous mycobacteria such as the mycobacterium avium complex is possible especially in persons infected with suppressed immunity such as the HIV infected. While this will not seem like an advantage for the GeneXpert, microscopy too will depend on culture to precisely identify cases of infection with mycobacteria other than tuberculosis. In cases where infection is established to be due to mycobacteria other than tuberculosis the drugs a patient has to take are different from the normal first- or second-line regimens (Riello, Brígido, Araújo, Moreira, Goulart & Goulart 2016:[2]). Therefore, the point that GeneXpert rapidly identifies species belonging to the MTB complex is still important although its role is limited in case of infection with non-tuberculous mycobacteria.

1.2.3 GeneXpert vs culture and phenotypic drug sensitivity testing

Compared to culture and DST, GeneXpert provides the advantage that it is rapid. In fact, if one considered the time to diagnosis for one patient then it would give results in two hours as opposed to culture and DST which would provide for the same patient in at least three weeks at best. A discussion can also be entertained about the simplicity of performing GeneXpert testing. The hands-on part of the procedure involves a user only having to mix one volume of sputum to two volumes of a proprietary reagent called ‘sample reagent’. The user will shake the mixture of sputum, wait for ten minutes and shake again. The reagent will liquefy the sputum so that five minutes after the second shaking, the user can load the liquefied sample into a special multi-chamber cartridge and load into the GeneXpert instrument. The GeneXpert instrument will carry out all the remaining steps of amplification and identification of MTB automatically within two hours (MSF 2017, WHO 2014:viii–ix, Steingart et al 2014:[4]). The steps for performing GeneXpert are so simple that even a lay health worker can be trained to carry out the testing without compromising the quality of testing.

In addition to simplicity GeneXpert also has the advantage of requiring less stringent measures for biosafety. In fact, WHO (2012:21) have recommended that GeneXpert can be performed under the same biosafety conditions as smear microscopy. But, for culture and DST a biosafety level 3 laboratory is needed to ensure that operators are safe while they conduct testing. It is partly due to these less stringent biosafety requirements that GeneXpert testing has perhaps more coverage than culture and DST.

Culture and DST are relatively complex techniques requiring well trained and experienced laboratory technicians and relatively more expensive Biosafety Level (BSL) III facilities to be carried out (WHO 2012:28). For instance, processing a sample for culture and DST involves decontamination whereby a laboratory technician will mix equal volumes of sputum with sodium hydroxide. The mixture is then shaken mechanically and allowed to react for twenty minutes after which the laboratory technician will add phosphate buffered saline to stop the reaction. Then the stopped mixture is separated by a refrigerated centrifuge. After centrifugation the laboratory technician will pour out the supernatant of the mixture and re-suspend the sediment in saline at which point the sample is ready to be inoculated onto appropriate media. All these steps must be performed in a class II biosafety cabinet.

Problems that may occur during these steps depend on the experience of the lab technician and they include possible loss of the mycobacteria due to over-decontamination, or loss of the mycobacteria due to survival of other fast-growing bacteria (GLI 2014:[32–34]).

DST is a second step following successful growth of mycobacteria in culture whereby the lab technician will inoculate mycobacteria in a series of drug free and anti-tuberculosis drug containing media to compare their growth characteristics. Thus, culture and DST are relatively more experience, equipment and biosafety facility intensive techniques when compared to GeneXpert and smear microscopy (WHO 2018b:2, 3). Perhaps this is why Joloba et al (2013) observed that culture and DST techniques are available only in limited locations within a national tuberculosis laboratory network in Africa.

This is not to suggest that there are no difficult conditions for implementing GeneXpert. However, the constraints for implementing GeneXpert are relatively easier for national tuberculosis control programs to overcome. For instance, GeneXpert operates below environmental temperature of 30^{o}C and thus requires installation of air conditioning in some hot places. However, in its defence, GeneXpert is not the only laboratory instrument which requires such operating conditions. One can speak also about the need for stable and backed up electrical supply which is ensured by installing an uninterruptible power supply (MSF 2017, WHO 2014:9). Previously GeneXpert installation also required the availability of considerable space to store the reagents which were packed in large boxes containing 60 cartridges. In the experience of the researcher, Cepheid (the manufacturer of GeneXpert) have solved this constraint by introducing a new packaging format of 50 cartridges per box, which takes less space.

1.2.4 Possible uses of GeneXpert data

The role of GeneXpert in tuberculosis control is well established. This role can be thought of at two levels which are first at individual case detection and second at a public health level, specifically surveillance. The use of GeneXpert for detection of TB is premised on recommendations first made by the WHO in late 2010. They constituted of a strong recommendation for the use of the GeneXpert as an initial rapid diagnostic test for TB in persons with presumptive TB who are either HIV positive or at high risk of having multiple drug resistant TB. In contexts with low HIV and/or low risk for MDR a conditional

recommendation was issued to follow up positive microscopy with GeneXpert dependant on availability of resources. The WHO updated the recommendations for use of GeneXpert in May 2013 (WHO 2011:10; 2013a:38, 39). These policies that regulate the use of GeneXpert for detection of TB and rifampicin are discussed further in sections 2.8 and 2.9.

The integration of GeneXpert into the surveillance framework for TB control may be traced to 2013 when the WHO updated case classifications to incorporate TB diagnosed through molecular methods including GeneXpert. For instance, instead of classifying cases based on smear status, the updated case definitions of 2013 simply refer to “bacteriologically confirmed cases”, which accommodates the various methods of biological detection of TB including GeneXpert (WHO 2013b:3).

In general, the WHO defines surveillance as the continuous systematic collection, collation analysis and presentation of health information for the purposes of planning, implementation and evaluation of health programs. Specifically, for TB control the WHO recommended a case-based surveillance system with monitoring of outcomes for cohorts. In this framework surveillance information was intended to be used for identifying gaps in program implementation, identify which interventions work thus should be continued and guide selection of research topics and provide supporting evidence for how the program may allocate resources going forward. Knowing that the 1994 framework for effective TB control was based on smear microscopy and culture one can conclude that the 2013 update of case definitions was important for integrating GeneXpert into the TB surveillance framework (WHO 1994:4, [s.a.]).

1.3 STATEMENT OF THE RESEARCH PROBLEM

In 1994, the WHO in collaboration with the International Union Against Tuberculosis and Lung Diseases (the Union), started an ambitious project to measure the size of the problem of anti-TB drug resistance using standard methods. The project was called the global project on anti-TB drug resistance surveillance. At the same time a supra national reference laboratory network was also established to ensure that laboratories participating in the global project were quality assured through proficiency panels on drug sensitivity testing.

Ideally all WHO member countries should be submitting data on TB drug resistance through continuous surveillance systems. Nevertheless, while WHO member countries build capacity for implementing continuous surveillance systems contributions to the global through special nationally representative surveys and sentinel surveillance are also acceptable.

However, a review of twenty years of the global project by Zignol, Dean, Fazlon, Van Gemert, Wright, Van Deun, Portaels, Laszlo, Espinal, Pablos-Méndez, Bloom, Aziz, Weyer, Jaramillo, Nunn, Floyd and Raviglione (2016:[1083]) showed that some member countries especially LMIC are lagging behind in the submission of anti-TB drug resistance data. In this context, lagging behind refers to either not having any baseline data at all or not having repeat surveys against which the baseline can be compared. As for Swaziland, the first drug resistant TB survey was carried out in 2009 (Sanchez-Padilla, Dlamini, Ascorra, Rüsch-Gerdes, Tefera, Calain, De la Tour, Jochims, Richter and Bonnet (2012) and to the researcher's a second one was planned for 2017/18.

Lagging behind in submitting data to the global project is a problem for TB management both at country and global level because national programs where there is no surveillance of anti-TB drug resistance lose the benefit of such data for planning to address MDR in their country. In addition, in the absence of surveillance data a national program may not be able to gauge their performance at least in terms of impact on drug resistance. On a global level, WHO and other partners may not be able to give national programs where surveillance data is missing targeted technical guidance to improve control of drug resistance. It is imaginable as well that when the size of the problem of drug resistant TB in country is unknown then they may fail to advocate for certain financial resources as well.

In agreement with Joloba et al (2013), Zignol et al (2016:[1086]) identify laboratory capacity to perform DST is one of the reasons for countries to fail to submit drug resistance surveillance. However, the introduction in 2010 of GeneXpert means that at least rapid DST for rifampicin can be done in a laboratory with biosafety requirements like those of smear microscopy (WHO 2011:5, 11). Further, the massive scale up of GeneXpert in member countries through the HBDC program means that GeneXpert is widely implemented in member countries (WHO 2016b), sometimes even more than phenotypic DST. For an example Alame-Emane, Pierre-Audigier, Aboumegone-Biyogo,

Nzoghe-Mveang, Cadet-Daniel, Sola, Djoba-Siawaya, Gicquel and Takiff (2017:[2106]) report that in Gabon there is countrywide penetration of GeneXpert yet there is no TB laboratory that performs culture.

Given the technical possibility for GeneXpert to detect rifampicin resistance, its widespread implementation in Swaziland, and the green light from WHO that molecular methods can be used for drug resistance surveillance, this study seeks to describe rifampicin resistance according to GeneXpert in Swaziland from 2012–2016. The period 2012 – 2016 was selected at least because it marks the first five years of GeneXpert implementation. Moreover, five years was a reasonably long period to allow the researcher to discover any trends in the prevalence and patterns of rifampicin resistance over time.

1.4 AIM OF THE STUDY

This study aims to determine the epidemiological prevalence and patterns of Xpert MTB Rif resistance in Swaziland from the year 2012 through to 2016. This may be a first step in identifying the possible value of routinely collected Xpert MTB/Rif data in the development of a continuous system of Rifampicin resistance surveillance. In low to middle income countries where traditional anti-tuberculosis drug resistance surveillance is not always possible, the possibility of using routine Xpert MTB Rif data presents interesting prospects for TB control enhanced by surveillance data. At the same time the analysis of patterns of rifampicin resistance according to GeneXpert may present prospects of a rudimentary system of molecular classification of MTB strains circulating in Swaziland.

1.4.1 Research objectives

- To describe the prevalence of TB / Rif drug resistance according to person, place and time, in the Xpert MTB/Rif data of 2012–2016 in Swaziland.
- To describe the mutations patterns identified by Xpert MTB/Rif probes among patients diagnosed with TB from 2012–2016.

1.4.2 Research questions

- What was the prevalence of TB/Rif drug resistance from 2012 to 2016 with regard to person, place and time?
- What were the mutations patterns identified by Xpert MTB/Rif probes among patients diagnosed with TB from 2012–2016.

1.5 SIGNIFICANCE OF THE STUDY

It has been more than twenty years since the beginning of the Global Anti-Tuberculosis Drug Resistance study but, there are still many WHO member countries, especially Low to Middle Income Countries (LMIC), unable to submit data on anti-tuberculosis resistance. At the same time, it is fairly accepted that knowledge of molecular epidemiology of Tuberculosis drug resistance is a key factor of successful TB control. Yet in many low to middle-income countries there is a paucity of data on anti-tuberculosis drug resistance, let alone, data on molecular epidemiology.

The current study used data from GeneXpert which is widely implemented in LMIC, to describe proportions of rifampicin resistance and the probes in which rifampicin resistance conferring mutations were located in Swaziland. This is a starting point for understanding both occurrence and distribution of rifampicin resistance and the underlying molecular mechanisms. In countries where Xpert MTB/Rif is implemented at national or other wide administrative level, the prospect is that its data can be used to inform policy makers about proportions of Rifampicin resistance among new and previously treated diagnostic cases.

This data may be useful for identifying localized outbreaks and hotspots of rifampicin resistance. The ability to detect outbreaks and hotspots is critical for focusing efforts of the national tuberculosis control program. For instance, areas identified as hotspots for rifampicin resistance may be candidate areas for focused intensified case finding and or health awareness campaigns and or implementation of GeneXpert at lower levels of the health care system. Thus, the findings of this study suggest new simple data driven approaches for TB control in LMIC countries where GeneXpert has been implemented widely.

The study was also significant for Swaziland, where it was carried out. It is possible that through routine reporting some information was already known about rifampicin resistance detection by GeneXpert. However, the current study analysed rifampicin resistance proportions, revealing trends in rifampicin resistance over a period of five years. Information about trends is useful for identifying where Swaziland stands in terms of TB control and assessing the effectiveness of TB control efforts by the national TB program. The study also described the occurrence of TB according to person, place which the national TB program can use to identify which persons are most affected by tuberculosis, in which places and how has this been evolving over time. This information not only extends what is known about TB in Swaziland but can be used to directly inform policy and plans of action against TB.

For instance, the national TB control program could use this study's finding that the smallest proportion of patients with rifampicin resistant TB reside in Lubombo to check if there is a need to increase access to GeneXpert testing in that region. Similarly, in Manzini where the highest proportion, the national TB control program may check whether more testing centres are needed.

1.6 DEFINITIONS OF KEY TERMS

1.6.1 GeneXpert

GeneXpert' refers to a platform for rapid real-time detection of organisms using real time polymerase chain reaction (Lawn & Nicol 2012:[4]). The word platform is used to signify that the GeneXpert modules can take and process cartridges for the detection of other diseases. The platform was developed initially for detection of anthrax in US mail sorting systems. In 2006 Foundation for Innovative New Diagnostics (FIND) worked with the University of New Jersey Medicine and Dentistry to develop a cartridge-based test used on the GeneXpert platform to simultaneously detect MTB and rifampicin resistance, called Xpert MTB/Rif (Lawn & Nicol 2012:[3]).). Thus, the rapid molecular test for TB based on GeneXpert is called Xpert MTB/Rif. However, it is common to find the terms used interchangeably and it shall be used as such in this study.

1.6.2 New patient

Refers to a patient who has never taken anti-TB treatment or has taken anti-TB treatment for less than 30 days (WHO 2013b:4). In this study, new patient refers to a patient who at the time of diagnosis, for the period under study 2012–2016, had never taken anti-TB treatment or had taken anti-TB treatment for less than 30 days.

1.6.3 Previously treated patient

Refers to a patient who at the time of diagnosis, for the period under 2012–2016, had received anti-TB treatment for at least 30 days in the past (WHO 2013b:4). This group of patients can also be subdivided into relapse, treatment after failure, and treatment after loss to follow up. In this study, all the sub-categories will be referred to simply as previously treated patients. The reason was that data on the various sub-categories was expected to be incomplete.

1.6.4 Molecular probe

Probe refers to a fluorescein tagged DNA sequence, complimentary to a part the DNA of drug sensitive mycobacterium tuberculosis used to detect the presence or absence of its complement in the GeneXpert PCR cycle. GeneXpert includes five probes (A–E) (Lawn & Nicol 2012:[4]).

Rifampicin resistance refers to the presence of at least one mutation in the 81-base pair region of the rpoB gene of mycobacterium tuberculosis as interpreted by Xpert MTB/Rif software (Lawn & Nicol 2012:[4]). In some countries where traditional culture and drug resistance testing are not widely available the possibility of using routine GeneXpert data presents interesting prospects for evidence-based tuberculosis control.

1.6.5 Participants, respondents, observations or GeneXpert records?

Polit and Beck (2012:48) differentiate persons cooperating in a research study on the basis of the type of study in which they are involved. Thus, they refer to a person as a subject or participant when the study in which they are cooperating is a quantitative study. Alternatively, Polit and Beck refer to persons taking part in a qualitative study as

participants, in formants or key informants. Babbie (2014:202) makes a distinction between levels of participation when he says that respondents provide information about themselves while informant provide information about the group about which a researcher seeks to make conclusions. The idea of distinguishing between levels of participation is also echoed by Roller (2013), who views a respondent a person who simply answers the questions of the researcher. This would include persons who respond to questionnaires. On the hand, Roller sees a participant as having more active engagement with the researcher. Such engagement may include expanding on questions asked by the researcher, willingly agreeing to be observed and engaging other participants as would happen in a focus group discussion.

In this study, persons whose records were included in the study had initially been called participants. This is in agreement with the idea of Polit and Beck that persons cooperating in quantitative study can be called participants. At the same time, it is also clear that the present study recruited patient records. For this reason, the better advice of examiners was to refer mostly to them as GeneXpert records. However, in some cases the researcher found it appropriate to discuss the record instead of the participant. In such cases the record was referred to as an observation. According to SAS ([s.a.]), an observation is a row in a data set. An observation has either one data value or a missing value indicator for each variable. This definition made it intuitive for the researcher to use the term ‘observation’ when discussing missing values for certain variables like age and gender.

1.7 RESEARCH DESIGN

This was a descriptive correlational study. This study design was chosen because the primary question of this epidemiological study was to determine proportions of rifampicin resistance. Proportions of rifampicin resistance equate to prevalence of rifampicin and indeed in the context of this study the terms could be used interchangeable. On the other hand, the epidemiological study design best suited to calculate prevalence is the cross-sectional design. The study also has an analytical component which is why the study design was identified as descriptive correlational.

1.8 THEORETICAL FOUNDATIONS OF THE STUDY

1.8.1 Research paradigm

Polit and Beck (2012:11, 12) and Babbie (2014:33) concur on the idea that a research paradigm is the lens used by a researcher to look at and understand observations. Paradigms can be seen as a central hub with spokes that link to other parts of a study. For instance, Babbie (2014:33) makes a connection between paradigms and theories when he states that it is paradigms that determine which theories make sense and which ones do not. In social science, the two major categories of paradigms are positivism and constructivism. As Babbie (2014:22) mentions a researcher using a positivist paradigm would most likely employ deductive reasoning to test out a theory. Alternatively, a researcher employing a constructivist paradigm would be more comfortable to use inductive reasoning to make sense of her or his observations.

Polit and Beck (2012:12), on the other hand, link paradigm to basic philosophical questions and methodology. The basic philosophical questions address the two concepts of ontology and epistemology. Ontological questions are about the nature of reality, specifically whether there is one reality or multiple realities. In other words, the ontological questions ask whether there is an objective reality that can be observed regardless of the values of the researcher or is the reality out there constructed socially by individuals. A researcher espousing positivist traditions would most likely assert that there is an objective reality out there which can be described objectively. By contrast the researcher espousing constructivist traditions would be more comfortable imagining a reality which is socially constructed, with different individuals assigning different meanings.

Epistemological questions are about knowledge, its nature and mode of acquisition. Thus, as Polit and Beck (2012:12) mention epistemological questions ask what methods can be used to obtain knowledge. A researcher with positivist leanings will use methods that emphasise the collection of specific quantifiable concepts. As well, the research methods used may involve fixed, pre-specified designs with tight controls over context. This description of methods is synonymous with quantitative research methods.

By contrast a researcher with constructivist leanings will use research methods that focus on the totality of a phenomena. The data she / he collects may be subjective and non-

quantifiable and the methods used emergent rather than fixed. This description is synonymous with qualitative research methods.

This study was based in post positivist research traditions. As positivism and post positivism can be misunderstood, a brief history of positivism is given below, followed by the differences between the two. Babbie (2014:34) and Polit and Beck (2012:12) give somewhat differing but complementary accounts of the history of positivism. For Babbie (2014:34) it was Comte (1798–1857) who coined the positivist research paradigm. As Babbie (2014:34) narrates, Comte believed that there were three ages of human history namely theological, metaphysical and a third where religion and metaphysics were replaced by positivism. Positivism was so called, because for Comte the whole idea that scientific truths could be positively verified through empirical observation presented a new hope for humanity. Especially considering, in his view that previously humans were limited to understanding truth through only religion and metaphysics.

Polit and Beck (2012:12) complement the account of the development of the positivist tradition when they mention that positivism also benefited from the works of Locke, Newton and Mill. Polit and Beck (2012:12) describe the basic assumptions of positivism as including the belief that there is an objective reality that can be studied objectively. Further, Polit and Beck (2012:12) mention that positivists are also deterministic because they believe that the reality that is out there is ordered, and there are antecedent causes.

Post positivism though sounding related to positivism is a rejection of positivism. Specifically, the post positivist while still believing that there is a reality out there is aware and accepts that it is impossible to be totally objective. Nevertheless, objectivity remains an important goal for post positivists so that they try to be as neutral as possible. As a result, post positivists seek probabilistic evidence which represents what a reality is most likely to be.

Post positivism was a suitable research paradigm for this study because the study sought to determine the epidemiological prevalence of rifampicin according to GeneXpert. The findings of the study thus represent what the epidemiological prevalence is most likely to be given the limitations of methodology, participant selection and missing data.

1.8.2 Theoretical framework

Babbie (2014:33) characterizes the function of theories simply as to explain research findings. Polit and Beck (2012:126, 127) offer an extensive definition of theories as abstract generalizations that systematically explain the inter-relationships between phenomena. Further, Polit and Beck (2012:126) also describe a more general use of the term theory as used to refer to broad characterization that describe a single phenomenon.

According to Polit and Beck (2012:127) concepts are the basic building blocks of a theory. The relationships between concepts are indicated by propositions. Other terms that may be invoked when speaking about theories include principles which may explain why a certain phenomenon works the way it does and premises which state the basis on which propositions are made.

1.8.3 Social construction of technology theory

Social construction of technology (SCOT) is a theory situated in the field of science and technology studies. Its roots can be traced to the mid-80s where it emerged as a challenge to then mainstream theory of social shaping of technology (Pinch & Bijker 1984). While the social shaping of technology theory proposed that in the development of technology users were but a passive group of consumers, SCOT argues that social factors can affect the development of technology. SCOT places users at the centre of technology development as one of the relevant user groups. Particularly, that different users, assign different meanings to a technology and that as a result users, other relevant social groups and developers are involved a tacit negotiation which sees technology evolve and hopefully finally mature. The main concepts of SCOT include interpretive flexibility, social groups, closure and stabilization and wider context.

1.8.3.1 Interpretive flexibility

Pinch and Bijker (1984:[411]) propose that the development of technology is a multidirectional nonlinear process in which different user groups assign different meanings to the technology. This phenomenon is called interpretive flexibility and potentiates many possible outcomes of the technology to be developed with the final outcome being a result of negotiations between user groups (Pinch & Bijker 1984:[419]).

When applied to the development of GeneXpert one can imagine relevant social groups to include:

- clinicians who required a reliable rapid technology for detection of TB drug resistance and other diseases
- funders who required an affordable technology that can be scaled
- epidemiologists and who required the technology that can be incorporated into surveillance systems

Requirements of clinicians to have a rapid reliable technology for detection of TB and drug resistance may be credited for the development of the GeneXpert assay from a platform that previous was for rapid detection of anthrax. Moreover, GeneXpert has been developed for thirty-five other pathogens that include methicillin resistant staphylococcus aureus, chlamydia, gonorrhoea, and flu virus (Cepheid 2018a). Further, the revision of GeneXpert software and cartridges described in section 2.7.5.5, can be seen as the further negotiation between users and developers. At the same time interpretive flexibility can be used to understand the choice to have rifampicin resistance as the proxy marker for MDR TB (see section 2.6.1.3). Having one drug as a proxy might also be a move that keeps the technology affordable and hence scalable. As this study, used GeneXpert data for rifampicin resistance it can be considered that this study fits in the user group of the epidemiologists who seek ways to incorporate GeneXpert technology into existing surveillance mechanisms.

1.8.3.2 Closure and stabilization

Pinch and Bijker (1984:[424]) mention negotiations for the final outcome of a technology continue until all who are concerned with the technology are satisfied with it. Closure is achieved when no more revisions are possible, and the technology takes its final form. Besides the revision of GeneXpert described in section 2.7.5.5, Chakravorty, Simmons, Rowneki, Parmar, Cao, Ryan, Banada, Deshpande, Shenai, Gall, Glass, Krieswirth, Schumacher, Nabeta, Tukvadze, Rodrigues, Skrahina, Tagliani, Cirillo, Davidow, Denkinger, Persing, Kwiatkowski, Jones and Alland (2017) describe GeneXpert Ultra which is new software and cartridge of GeneXpert aimed at increasing sensitivity of detection. The researcher thus can only speculate that GeneXpert is not yet in the closure and stabilization stage.

1.8.3.3 Wider context

Wider context refers to the social, cultural and political environment in which a technology is developed. The wider context may affect a technology directly or indirectly. For instance, for GeneXpert one may recognize that the need for rapid diagnosis and early initiation of appropriate treatment of TB patients, more so in LMIC might have been a factor in the rapid uptake of GeneXpert as a technology. At the same time this was enhanced by the availability of concessionary prices for the most affected countries through the High Burden Disease Countries program of Cepheid (Cepheid [s.a.]b, WHO 2016b). Research can also be considered as part of the wider context in the sense that it was through evidence from studies that GeneXpert was formally recommended by the WHO (WHO 2011, 2013a).

1.8.4 Application of the social construction of technology

This study employs the social construction of technology to understand how GeneXpert data can be used to solve the problem of missing information about rifampicin resistance from LMIC. The results of this study thus contribute to an ongoing negotiation about how the data can be made more available and usable to satisfy this purpose. The application of SCOT into this study means that the theory formed the basis for elements of the study that include the problem, purpose, research questions and significance. Moreover findings were also interpreted in light of SCOT.

1.8.4.1 Application of the social construction of technology to the problem

One of the main elements of the dilemma whose solution this study sought to contribute was the lacking of anti-tuberculosis resistance surveillance data from certain WHO member countries, especially LMIC. At the same time, there was a recognition that in addition to producing results for clinical management of patients, GeneXpert generated deep machine data which included the data on the specific probes in which the mutations lay.

In terms of SCOT, the researcher fell into a relevant social group whose concern was to have anti-tuberculosis surveillance data. In keeping with the process of interpretive

flexibility, the researcher viewed the GeneXpert not only in terms of rapid results for rifampicin resistance but also in terms of using the data to answer to the problem of missing surveillance data.

1.8.4.2 Application of the social construction of technology to the purpose

The study's aim of determining the epidemiological prevalence and pattern of rifampicin resistance in Swaziland is in itself a process of social construction. The social construction processes in this case proceeded through an overarching question about how GeneXpert could answer a gap in the submission of anti-tuberculosis resistance data by some WHO member countries. Consequently, the researcher posed questions about the prevalence of rifampicin resistance by person, place and time and the patterns of resistance according to GeneXpert. By answering these questions the researcher essentially showed that GeneXpert could also be used for surveillance of rifampicin resistance. This also is social construction of the artefact which is GeneXpert.

1.8.4.3 Application of the social construction of technology to the signifance

The researcher understands SCOT as broadly referring to a process whereby one artefact may be viewed differently by various relevant social groups. Implicit in this understanding is the active role taken by the members of a relevant social group to present their alternative view of the artefact. Essentially this is achieved by answering the question of "so what?", which links directly to the significance of the study. The researcher views the significance of this study as being at two levels. at the global level WHO member countries could develop rifampicin resistance surveillance systems based on GeneXpert. At the country level Swaziland health authorities could use the findings of the study to analyse what happened to the epidemiological situation of TB in the first five years of implementation of GeneXpert.

1.9 RESEARCH METHODS

A two-stage sampling procedure was employed to select GeneXpert records. In the first stage nonprobability purposive sampling was used to select nine laboratories. Then in the second stage random sampling was used to select 50% of all GeneXpert positive records. Data was entered in Microsoft Excel and cleaned. SAS Studio version 9.04M6

was used to calculate frequencies with 95% confidence intervals as well as proportions of resistance by year, age group, region, and gender. A logistic regression model was used investigate association between each of age group, gender and region and development of rifampicin resistance.

1.10 SCOPE OF THE STUDY

The study focused on diagnostic pulmonary TB patients who submitted sputum between and including 2012 and 2016. Other samples are excluded even though MTB may have been detected.

Conclusions drawn for the 2012 may be limited in that for most of that year Xpert MTB/Rif was not implemented in some health centres. From 2013 Xpert MTB/Rif data had been implemented to cover the whole country.

1.11 STRUCTURE OF THE DISSERTATION

Chapter 1: The first chapter contains the introduction to the study. It provides the context, the statement of the problem and how this study seeks to contribute to the solution. The aim, objectives, significance and scope of the study are also stated herein. The chapter also gives highlights of the research design and methods. The chapter ends by giving the reader the structure of remaining chapters of the study.

Chapter 2: This chapter contains a review of what is already known about the uses of GeneXpert. The review is focused on the uses of GeneXpert because the question addressed by this study was about the uses of GeneXpert data. The chapter is structured so that the principles of how and why GeneXpert generates its data are reviewed first followed by the major application of GeneXpert, which is in detection of tuberculosis and rifampicin resistance for clinical management of patients. Then other uses of GeneXpert, its by-products and data are reviewed. It is in the context of uses for GeneXpert that this study seeks to extend the body of knowledge.

Chapter 3: This chapter expands on the research design and methodology. While design is overall covering all aspects of how the study obtained its data that answered the questions of this study, methodology focuses on the individual aspects like sampling, data collection, data cleaning, storage, and analysis.

Chapter 4: This chapter reports on the findings of the study. To link the processes of data collection and analysis, the chapter begins by presenting a patient flow. The patient flow is important for readers to visualize all the steps in which potentially eligible patients were excluded from the analysis. This way readers can make better informed judgements of what biases may affect the findings of the study. The findings are presented in a systematic fashion which begins by presenting demographic data of patients, followed by proportions of rifampicin resistance by age group, gender and region of origin. For rifampicin resistant strains the frequencies of the probes in which mutations are located are presented. Lastly, the chapter also presents the results of logistic regression model of how age group, gender and place may influence development of rifampicin resistance.

Chapter 5: This chapter presents an interpretation and discussion of the results presented in Chapter 4. The chapter attempts to make sense of findings by comparing findings with other studies in and outside Swaziland. Some recommendations are also suggested which will be developed in the chapter. The chapter then points out the limitations of the present study before making conclusions on the main questions of the study.

References: This section contains an alphabetical list of sources referred to in this study.

Annexures: This section contains the annexes to the study.

1.12 SUMMARY

This chapter familiarized the reader with the main directions of the study by giving a brief background to the problem, a problem statement and how this study proposed to solve the problem. Specifically, there is a gap in data submitted to the global project on anti-tuberculosis resistance. LMIC countries are among the countries which are unable to submit anti-TB drug resistance data because they lack laboratory capacity for traditional DST. This study explores the possible value of GeneXpert data for providing at least rifampicin resistance data in LMIC. This study was concerned with the uses of GeneXpert which is why in Chapter 2 the literature reviewed is that about the principles of GeneXpert, its principal use in detection of TB and rifampicin resistance and the possible uses of GeneXpert data and by products.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The previous chapter set the context for the study, identified the problems and the general principles of how this study addresses these problems. This chapter presents a review of GeneXpert as a method of diagnosis for TB and rifampicin resistance. As the main theme of this study was about the use of GeneXpert for rifampicin resistance, the literature reviewed here addresses technical principles behind the operation of the GeneXpert for detection of TB and rifampicin resistance, regulatory frameworks within which GeneXpert is used for clinical management of patients and the possible uses of GeneXpert data and its by-products.

The technical principles are presented first not only as an overview of why and how the technology works but also a review of how GeneXpert data, which was the subject of this study, is generated. The main concepts reviewed include the target sequence, the design features of the GeneXpert cartridge and real time Polymerase Chain reaction (PCR) employing molecular beacons. The review then moves on to the policy framework regulating the use of GeneXpert for diagnosis of TB and rifampicin resistance which are the WHO policy on the use GeneXpert of 2010 and its update of 2013. These two policies are reviewed here because GeneXpert is most commonly used in the diagnosis of TB and rifampicin resistance. As this study seeks to describe the use of GeneXpert data, it seems useful to review the basis for its most common use. Lastly the review makes a survey of other uses of GeneXpert outside of the main use of routine diagnosis of TB and rifampicin resistance. The adaptation of GeneXpert for other purposes is the context in which this study suggests the use of GeneXpert data for rifampicin resistance.

2.2 THE 81 BASE PAIR REGION IS THE TARGET SEQUENCE FOR GENEXPERT

The GeneXpert applies real time PCR to identify both TB and rifampicin resistance in clinical specimens. PCR is an enzymatic process in which a specific region of Deoxyribonucleic Acid (DNA) is replicated many times to produce many copies (Butler

2012). The variation of PCR employed in GeneXpert is called real time because the products of PCR are detected as they are produced (Clark & Pazdernik 2013:182, Lawn & Nicol 2012:[3]). The specific region of DNA is which is to be amplified is sometimes also referred to as the target sequence. The product of PCR is called the amplicon (Butler 2012).

PCR is very sensitive for diagnosis of TB, because tiny amounts of target sequence present in the sample can be amplified to millions of copies which are detectable. Butler (2012) attests to this when he states that under conditions of perfect amplification efficiency almost a billion copies of amplicon would be produced in 32 PCR cycles. A PCR cycle consists of the three steps namely denaturation, annealing and elongation.

Denaturation takes place at temperatures around 90^{o}C. At this temperature the hydrogen bonds that hold DNA strands together are disrupted resulting in single strands of DNA. Temperature is then dropped to between 50–60^{o}C in the second stage of the PCR cycle. This allows primers to attach to the single stranded DNA. Primers are short sequences of single stranded DNA that are complementary to the DNA sequences that flank the target sequence. Finally, an enzyme called DNA polymerase completes the PCR cycle by adding nucleotides to the DNA fragment initiated by the primers. Typically, 30–40 cycles constitute a PCR run (Clark & Pazdernik 2013:166–168).

The target sequence for GeneXpert is the 81 base pair region of the rpoB gene. Target sequence here, refers to a specific region of DNA which GeneXpert amplifies in order to detect TB and rifampicin resistance. The rpoB gene codes for an enzyme called Ribonucleic Acid (RNA) polymerase. Brandis, Wrande, Lijas and Hughes (2012:[142]) describe RNA polymerase as a 400kDa enzyme composed of five subunits. They are one αα homodimer, one ββ′ heterodimer and ω subunit. The catalytic centre of the RNA polymerase is in the β subunit. The role of the RNA polymerase is to catalyse the elongation of messenger RNA. Rifampicin binds to the β subunit, in so doing blocking the path for elongating RNA when it is 2–3 nucleotides long. Once that happens elongation of messenger RNA is inhibited thus resulting in the death of MTB. However, some mutations in the rpoB gene result in rifampicin resistance as described in paragraphs below.

2.3 RIFAMPICIN IS KEY IN TUBERCULOSIS TREATMENT

Rifampicin is such an important component of first line TB treatment because it is active against both rapidly and slowly metabolizing MTB bacilli. Also, rifampicin treatment is known to have less side effects on patients compared to second line drugs for (Arbex, Varella, De Siqueira, & De Mello 2010:[631]). However, ever increasingly resistance to this drug is now being experienced. Moreover, it is also known that rifampicin resistance occurs in conjunction with resistance to other anti-tuberculosis drugs. This importantly together with resistance to isoniazid which is the other most effective drug for treatment of tuberculosis (Palomino & Martin 2014:318]). The simultaneous occurrence of resistance to both isoniazid and rifampicin whether alone or in conjunction with other first line anti-tuberculosis drugs is the defining criteria for multidrug resistant (MDR) TB (WHO 2018c). In recent years the emergence of MDR TB has become a problem of public health concern, threatening to cripple or even reverse the gains so far achieved in TB control. For instance, the WHO reports that in 2016, of the 10.4 million incident cases of TB, 600 000 were MDR (WHO 2017b:1).

2.4 MECHANISMS OF RIFAMPICIN RESISTANCE

2.4.1 Innate mechanisms of anti-tuberculosis drug resistance

Resistance to anti-microbial drugs in mycobacteria involves innate and molecular mechanisms. Innate mechanisms include the cell wall which contains mycolic acids that limit the entry of drugs into the bacilli. As Somoskovi, Parsons and Salfinger (2001:[164]) point out efflux pumps are also part of innate mechanisms and they work by pumping drugs out of the cytoplasm of the bacilli. In this way drugs affected by efflux pumps fail to achieve high enough concentrations to affect the bacilli. Innate mechanisms probably contribute little to anti-tuberculous drug resistance in reality but should be considered when the mutation behind resistance cannot be identified in the RRDR or other sites known for rifampicin resistance (Machado, Couto, Perdigão, Rodrigues, Portugal, Baptista, Veigas, Amaral & Viveiros 2012:[2]). Perhaps this is why some such as Dookie, Rambaran, Padayatchi, Mahomed and Naidoo (2018:[1139]) simply single out molecular mechanisms as being the primary way in which resistance occurs in mycobacterium tuberculosis. In this review no more attention will be paid to innate mechanisms.

2.4.2 Molecular mechanisms of anti-tuberculosis drug resistance

As early as the 1970s work by Woodley, Kilburn, David and Silcox (1972:[247]) had raised the assertion that rifampicin resistance occurred in MTB species as a result of spontaneous mutations. So was the recognition that the presence of the corresponding antibiotic exerted selective pressure which encouraged the proliferation of drug resistant mutants. Mutations occur mainly the RRDR as the paragraphs below describe.

2.4.2.1 The role of the rifampicin resistance determining region

Although they acknowledge the accessory role of efflux pumps Machado et al (2012:[2]) suggest that molecular mechanisms play a more important role in mediating resistance to anti-tuberculosis drugs. Resistance has been shown to be present when there are mutations in genes that code for either the targets for anti-tuberculosis drugs or the enzymes that activate the drugs. Rifampicin resistance has been shown to be mediated by mutations that code for the RNA polymerase which is the target for rifampicin (Campbell, Korzheva, Mustaev, Murakami, Nair, Goldfarb & Darst 2001:[901]). Palomino & Martin (2014:[318]) state that mutations in the rpoB gene result in a conformational change that limits affinity for the drug. Such mutations have been shown to occur mainly in the 81 base pair region of the rpoB gene which codes for RNA polymerase. This region is also referred to as the hotspot for rifampicin resistance or alternatively as the rifampicin resistance determining region (RRDR).

2.4.2.2 Mutations outside the rifampicin resistance determining region

It is also known now that a small percentage of mutations occur outside the RRDR. But in certain geographical regions, strains whose rifampicin resistance is coded by mutations outside this RRDR may occur in higher than normal frequencies giving the impression that mutations outside the RRDR contribute more than a small percentage. A good example is that of Swaziland where Sanchez-Padilla, Merker, Beckert, Jochims, Dlamini, Kahn, Bonnet and Niemann (2015:[1181]) found that mutation I572F located outside of the RRDR was present in 38% of MDR strains obtained through a nationally representative drug resistance survey. This finding is significant in two ways. First, it demonstrates the disparate distribution of uncommon strains whose resistance is coded

outside the RRDR. Second the findings demonstrate some of the challenges still faced when detecting rifampicin resistance using new rapid molecular diagnostics. These challenges are discussed in more detail in section 2.5.4.

2.5 NUMBERING SYSTEMS FOR CODONS

In terms of the genome of mycobacterium tuberculosis two important numbering systems are to be kept in mind. These are the Escherichia coli and Mycobacterium tuberculosis systems. An important issue pointed out by Andre, Geominne, Cabibbe, Beckert, Mukadi, Mathys, Gagnuex, Niemann, Van Ingen and Cambau (2017:[170]) is that different systems for numbering the codons where mutations occur can make it difficult to correctly compare mutations across studies.

2.5.1 The Escherichia coli numbering

The Escherichia coli numbering system can be considered to have been popularized by Telenti, Imbodeni, Marchesi, Lowrie, Cole, Colston, Matter, Schopper and Bodmer (1993), when they determined mutations behind rifampicin resistance in 64 drug resistant strains from various geographical regions. In that seminal study Telenti et al (1993:650) used the Escherichia Coli numbering system to number codons perhaps implicitly endorsing this system of numbering, even suggesting that the amino acid sequence of the rpoB gene was highly conserved across several bacterial species. The reality as Andre et al (2017:[168]) point out is that similarity between Escherichia coli and Mycobacterium tuberculosis is only moderately high within the RRDR and drops significantly once outside the RRDR. This is not surprising given that Andre et al (2017:[168]) also point out that the rpoB genes of Escherichia coli and Mycobacterium tuberculosis are different in length. They are 4029 and 3519 base pairs respectively (EcoCyc [s.a.]; EPFL [s.a.]).

2.5.2 The mycobacterium tuberculosis complex numbering system

The mycobacterium tuberculosis complex numbering system takes into consideration the position of codons in relation to the beginning of the rpoB gene. Andre et al (2017:[170]) advocate for this numbering system as a consensus numbering system to improve understanding of the findings for different studies. The need to identify mutations causing

rifampicin resistance outside the RRDR is a compelling reason to adopt the mycobacterium tuberculosis numbering system. As mentioned earlier, similarity between the Escherichia coli and MTB rpoB genes is much lower outside the RRDR. This means that converting between the two reference systems becomes increasingly difficult once one begins to refer to codons outside the RRDR. However, rifampicin resistance coded by mutations outside the RRDR occurs albeit rarely.

2.5.3 Possible confusion comparing findings using different numbering systems

Andre et al (2017:[170]) erroneously substantiate the confusion that may occur when different numbering systems are used by citing the secondary analysis of strains from the 2009 drug resistance survey in Swaziland. Andre et al (2017:[170]) allege that Sanchez-Padilla et al (2015:[1181]) mistakenly conclude that that the mutation I491F (according to the MTB complex numbering system), is a novel mutation behind a significant proportion of strains with rifampicin resistance. While it true that Sanchez-Padilla et al (2015) use the MTB complex system as Andre et al (2017:[170]) claim the former are clearly aware of the Escherichia coli numbering system equivalent which they correctly state as I572F. Further, Sanchez-Padilla et al's (2015) concern is with the proportion of resistance attributed to I572F because in other places the mutation had only been responsible for a small proportion of resistant cases. More importantly Sanchez-Padilla et al (2015:[1181]) were concerned that if such a high proportion of mutations is coded outside the RRDR, then the use of GeneXpert for rifampicin resistance detection in Swaziland potentially results in significant underestimation of the true proportion of rifampicin resistant cases.

2.6 REVIEW OF THE PRINCIPLES OF GENEXPERT TESTING

This section discusses the principles employed by the GeneXpert beginning with justifying why the choice of the RRDR seems an appropriate choice for target sequence. The review bases on the seminal work of Lawn and Nicol (2012), because these two authors provide the most comprehensive description of the how and why GeneXpert works.

2.6.1 Factors for selection of the 81 base pair region as target

2.6.1.1 Possible high sequence homology across members of MTB

Lawn and Nicol (2012:[3]) commend the developers of GeneXpert for selecting the 81 bp region as the target for detection of rifampicin resistance. As earlier remarked by Telenti et al (1993:650) amino acid sequences in the RRDR are mainly conserved across bacterial species more so within the mycobacterium tuberculosis complex. Andre et al (2017:[168]) place the similarity between rpoB gene sequence of mycobacterium tuberculosis complex, mycobacterium leprae and mycobacterium kansasii to be in the region of approximately 88%–93%.

Given this high sequence homology across mycobacterial species one expects that across the 8 species that make up the mycobacterium tuberculosis complex, sequence homology is even higher. It then follows that, the RRDR is a good choice of target because it allows methods that are based on it to identify MTB and rifampicin resistance across differing species that comprise MTB complex.

2.6.1.2 The rifampicin resistance determining region harbours majority of rifampicin resistance conferring mutations

The RRDR is also a good target choice considering that most of the mutations coding for rifampicin resistance are located within the RRDR (Zaw, Emran & Lin 2018:[605]). As Brandis et al state (2012:[142]) most high-level mutations for mycobacteria occur in one of four regions of the β subunit. These are the N-terminal cluster, cluster I, cluster II and cluster III, which span codons 146–148, 507–534, 563–574 and 687 respectively. Interesting 95% of the high-level mutation are found in cluster I which happens to include the 26 codons between positions 507–533 which constitute the RRDR as identified by Palomino and Martin (2014:[318]). Mutations have so far been reported at positions 513, 516, 518, 522, 526, 529, 531 and 533 (Zaw et al 2018; Adikaram, Perera, & Wijesundera 2012:527; Prim, Schorner, Senna, Nogueira, Figueiredo, De Oliviera, Rovaris & Bazzo 2015:619–620 ; Bhembe, Nwodo, Govender, Hayes, Ndip, Okoh & Green 2014:[7, 8]; Dookie, Sturm & Moodley 2016:[3, 4]) .

In terms of frequency, it appears that mutations at positions 526 and 531 constitute most of the rifampicin resistance conferring mutations. However, there seem to be exceptions in the patterns seen particularly in South Africa and Swaziland. Dookie et al (2016:[3]) found that most strains (9/15), that were MDR had rifampicin resistance conferring mutations in the position 531 which would be consistent with findings elsewhere. However, they also found that almost all strains (21/28), which were XDR carried double mutations in positions 516 and 533. These findings raise more questions than answers regarding the precise mechanism by which a strain progresses to XDR. A commonly held view is that of progressive development of resistance starting from mono isoniazid resistance progressing to MDR and XDR consecutively. However, just from surveying the findings of Dookie et al (2016:[3, 4]) one gets the idea that perhaps there exists a different path to development of XDR. Otherwise, one of the double mutations should have been at position 531 which constitutes a majority of MDR strains.

The example of Swaziland has already been referred to above. In brief, molecular sequencing performed on strains from Swaziland revealed that 38% of rifampicin resistance conferring mutations were outside the RRDR meaning that GeneXpert would not be able to correctly determine rifampicin resistance in them.

While the studies above used some genomic sequencing methods to identify the mutations, the probes of GeneXpert can give an approximate idea of rifampicin conferring mutation in its deep data. Given that GeneXpert is more widely distributed than the sequencing methods, the use of deep GeneXpert data provides prospects of gaining an understanding about occurrence of rifampicin resistance and the mutations conferring it (WHO 2016b).

2.6.1.3 Rifampicin resistance predicts multidrug resistance better than Isoniazid

Another reason on which authors Lawn and Nicol (2012:[3]), Palomino and Martin (2014:[319]) and Dookie et al (2018:[1139]) concur is that choosing to detect rifampicin resistance is better predictor of multidrug resistant TB than when isoniazid is used as a target. As mentioned in section 2.2.1.1, MDR TB is marked by simultaneous resistance to isoniazid and rifampicin, so theoretically one could either aim to detect resistance to both drugs or detect resistance to one as surrogate of resistance to both. Rifampicin is a better predictor of MDR because mono rifampicin resistance is very rare. Often rifampicin

resistance occurs in conjunction with resistance to other anti-TB drugs, most commonly isoniazid (Palomino & Martin 2014:[318]). Mono isoniazid resistance on the other hand is quite common, perhaps explained by the fact that spontaneous mutations that can produce isoniazid resistance occur at comparatively higher rates (3.5×10^{-6}) than those producing rifampicin resistance (3.3×10^{-8}) (Velayati & Farnia 2017:154).

Besides, Lawn and Nicol (2012:[3]) explain that there are technical difficulties of relying on isoniazid alone to predict MDR. They refer to the findings of Piateki, Telenti, Murray, El-hajj, Jacobs, Kramer and Alland (2000:[105]). Piateki et al (2000:[105]) developed molecular beacon technology for identifying mutations coding for isoniazid and rifampicin resistance. Their findings showed that molecular beacon technology had 95% sensitivity for detection of rifampicin resistance compared to 85% for detection of isoniazid resistance. When one considers that the GeneXpert relies on molecular beacon technology it becomes clear why mutations coding for rifampicin resistance instead of isoniazid resistance are a better choice. Furthermore, Piatek *et al* (2000:[105,106]) also observed that the sensitivity of detection of rifampicin resistance was the same across strains obtained from a reference laboratory in Spain and those obtained from clinical specimens in New York. For isoniazid on the other hand sensitivity of detection of resistance was 96% among strains from the reference laboratory but only 44% among the clinical specimens.

2.6.1.4 The 81 base pair region is flanked by MTB specific sequences

Lawn and Nicol (2012:[3]) also mention that the 81 base pair region is a good choice of target sequence because it is flanked by MTB specific sequences. However, the GeneXpert does not probe for MTB specific sequences to determine the presence of MTB. Therefore, one wonders to what extent the presence of flanking MTB specific sequences influences the choice of target. All the same it remains that the RRDR is indeed a good choice of target sequence for the detection of rifampicin resistance and by proxy MDRTB. Having established the idea that the developers made a good choice in selecting to use the 81 base pair region as a target the discussion will now move on to describing how GeneXpert exploits the 81 base pair region to detect MTB and rifampicin resistance. In addition, the nature of deep machine data which is generated in the process and its possible uses are also be described.

2.7 OPERATING PRINCIPLES OF GENEXPERT

The process and principles applied by the GeneXpert in diagnosing TB can be broadly divided into two phases. The first phase comprises preparatory steps carried out by the operator outside the GeneXpert instrument while the second phase happens once the sample is loaded into the cartridge and placed inside the instrument as described below.

2.7.1 Hands on phase

Phase one is the hands-on segment where the operator prepares a clinical specimen appropriately so that it can be loaded into the GeneXpert. It involves mixing the specimen with a proprietary reagent in the ratio of 1:2 or 1:3 depending on whether the specimen is sputum or concentrated sputum sediment respectively. The sample reagent contains sodium hydroxide and isopropyl alcohol (Cepheid 2018b:[5]) These two reagents prepare the specimen by decontaminating and liquefying it. The operator is only expected to shake the specimen-sample reagent mixture at the beginning and at ten minutes of incubation. Five minutes after the second shaking the operator pipettes at least two millimetres of the liquified specimen into the GeneXpert cartridge (Cepheid 2012:[5–7]).

Liquification is important in preparing the specimen for PCR because it avails bacilli trapped in mucus for the next steps of specimen processing (GLI 2014:[19]; Wanger, Chavez, Huang, Wahed, Actor & Dasgupta 2017:90). There is also a technical reason why liquification of the specimen is important. That is, the GeneXpert uses a plunger rod to aspirate specific volumes and these volumes would be inaccurate if the liquified specimen is too viscous. In fact, whenever a liquified specimen is too viscous, then the GeneXpert will return error code 2008 (Baron 2016).

Decontamination is necessary to ensure that the procedure is safe for the operators. Banada, Sivasubramani, Blakemore, Boehme, Perkins, Fennelly and Alland (2010:3552–3555) demonstrated that during GeneXpert sample preparation more than 97% of the bacilli in the sample are killed by the end of the decontamination procedure. For this reason, the WHO recommends that GeneXpert be carried out under the same biosafety conditions as would apply to microscopy (WHO 2011:5, 11). Still some caution needs to be exercised and where possible this procedure must be done under at least a fume hood that can protect the operator from potential aerosols generated during the shaking at the

beginning and at 10 minutes. This is because in the evidence of safety cited above, the researchers determined a greater than log four killing at 15 minutes but according to the procedure the sample is shaken by the operator at 0 and 10 minutes. In any case where resources permit even the preparation of microscopy smears is being carried out in special inexpensive ventilated workstations such as the one designed by Angra, Coetzee, Eagleson, Feldman, Fernandes, Gilpin, Seng Goh, Jensen, Kreitein, Landy, Maryogo-Robinson, Nesby, Parsons, Ridderhof, Smithwick, Stotz, Timperi, Toney, Ugwu and Williams ([s.a.]).

2.7.2 In machine processing

Phase two takes place inside the plastic eleven chamber GeneXpert cartridge. The main elements of the multi-chamber cartridge include the multiple chambers, a rotary valve system, a central syringe barrel and a PCR reaction vial as shown in Figure 2.1. Below is a brief description of each of these main elements.

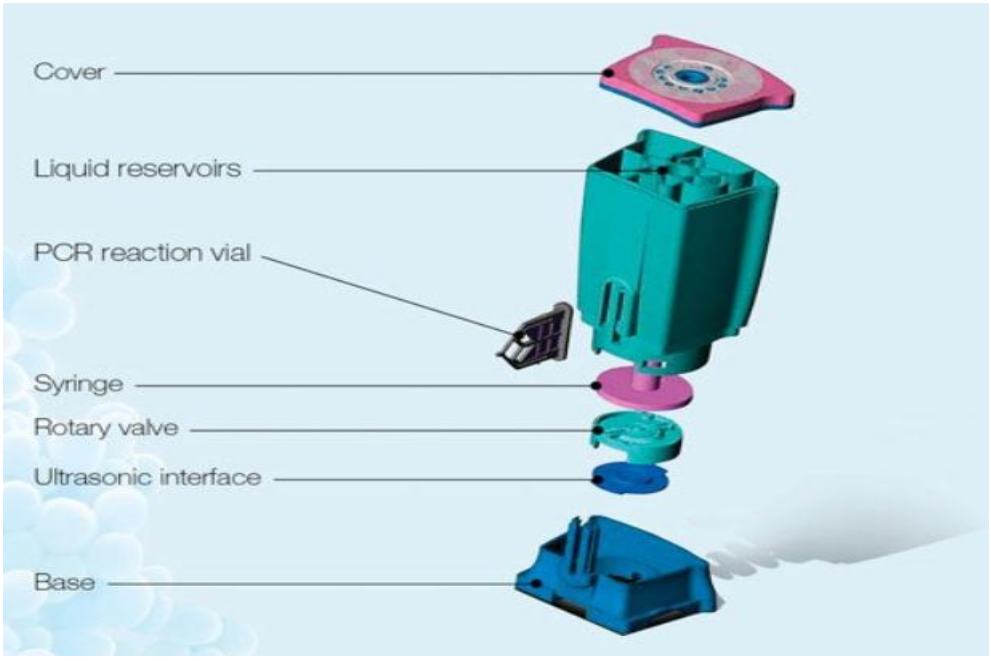


Figure 2.1 Main components of the GeneXpert cartridge

(Baron 2016)

2.7.2.1 Role of multiple chambers

The chambers of the cartridge contain the required buffers and reagents to extract DNA from the bacilli in the specimen and then amplify a 192 base pair fragment which includes

the RRDR. Some of the chambers contain reagents for specimen preparation while the other chambers have reagents for and are used for PCR (Baron 2016).

2.7.2.2 The rotary valve system and syringe

The rotating valve provides a way to move reaction mixtures from one chamber to the other. The GeneXpert instrument interacts with the cartridge through a plunger rod that inserts into the syringe barrel. In this way accurate small volumes of the reaction mixture can be aspirated and dispensed without a wet interface between the GeneXpert instrument and the sample. This so-called closed fluidics system eliminates the risk of cross contamination between samples, a problem which often can be challenging in conventional PCR (Soundiram 2012).

2.7.2.3 The polymerase chain reaction vial

The PCR reaction vial is surrounded by heating plates and optical detection blocks when inserted into the module. At the same time, it is connected to some of the cartridge chambers so that once sufficiently prepared, the sample is ported to the reaction tube. The heating plates facilitate PCR by heating and cooling up to the different temperatures required for initiating and processing polymerase chain reaction. Products of PCR are detected through the optical blocks surrounding the reaction tube in real time (Baron 2016).

2.7.3 Capture of mycobacterial cells

Capture of whole mycobacterial cells is one of the first processes undertaken by the GeneXpert on the liquified sample loaded into the cartridge. Blackmore, Story, Helb, Kop, Banada, Owen, Chakravorty, Jones and Alland (2010:[2496]) describe that through the plunger rod, GeneXpert first aspirates a 200 µL portion of liquified sample from the cartridge and mixes it with the sample processing control (SPC). The SPC is composed of spores of bacillus globigii which serve as an internal control for the process of amplification.

According to Baron (2016), the spores are a suitable process control because they are subjected to the same processes as the mycobacterial cells in the patient specimen. Also,

it is more difficult to extract DNA from spores than from mycobacterial cells. Thus, when the SPC is successfully amplified then it is sure that any mycobacterial DNA in the specimen would have been amplified as well. Baron (2016) also explains that the amplification of the SPC is multiplexed to that of MTB DNA in the specimen, such that runs where MTB DNA is not amplified will be positive for the amplification the SPC. This is a valid way to control for conditions of amplification because intuitively, if in a particular run DNA could be extracted from spores, then any MTB DNA would have been extracted from mycobacterial cells had they been present in the specimen.

The liquified specimen/SPC mixture is then aspirated through a sub-micron filter to capture SPC and any mycobacteria from the liquified specimen. Then an additional 1200 µL of the liquified specimen is again aspirated through the submicron filter.

2.7.4 Deoxyribonucleic acid extraction by ultrasonic lysis

In the next step DNA is extracted from the MTB bacilli and the SPC through ultrasonic lysis. The extracted DNA is eluted from the filter and mixed with master mix for the first PCR which amplifies a 192 base pair fragment which includes the RRDR. The reaction mixture is pushed into the reaction tube where PCR proceeds by following an initiation stage at 95^{o}C for 2 minutes followed by 16 melt/anneal cycles at 95^{o}C for 5s and 72^{o}C for 40s (Blackmore et al 2010:[2496]; Lawn & Nicol 2012:[4, 5]).

2.7.5 Heminested polymerase chain reaction

The product of the first PCR is the template for the second PCR. This is termed hemi nested PCR. Hemi nested PCR eliminates non-specific amplification thus increasing sensitivity of detection. It is designed in such a way that the first reaction amplifies a fragment that includes the target sequence flanked by other nucleotides that are not of interest. In the second reaction, primers are introduced that bind to the product of the first reaction. Non-specific binding is reduced because the majority of DNA sequences to which the second set of primers bind was produced in the first reaction Wanger *et al* (2017:247).

In the GeneXpert after the first reaction is completed, excess product is pushed out into a waste chamber and master mix containing primers for the 81 base pair region are

introduced in the PCR reaction tube. PCR proceeds similarly to the first reaction except that there are 45 melt/anneal cycles as compared to 16 for the first reaction (Blackmore et al 2010:[2496]).

2.7.6 Real time detection of polymerase chain reaction products

The detection of PCR products in real time is facilitated by the optical block which surrounds the PCR reaction tube. The identification of MTB and rifampicin resistance is based on the detection of fluorescence by the optical block. The GeneXpert uses a set 5 of overlapping probes spanning the entire 81 base region. The probes are molecular beacons, with a nucleotide sequence complementary to that of wild type mycobacterium tuberculosis (Baron 2016). Wild type mycobacterium tuberculosis refers to a strain of MTB which is sensitive to all first-line anti-tuberculosis drugs.

2.7.6.1 Molecular beacon technology principles

The idea of the molecular beacon is that if a desired target sequence is present in the products of PCR, then the molecular beacon will fluoresce. When the product of interest is absent in the PCR products there will be no fluorescence. In general, molecular beacons consist of a probe and two complementary DNA sequences attached to either ends of the probe. In native state, the complementary sequences hybridize to each other so that the probe takes the form of a stem and loop. A fluorescein is attached to the end of one of the complementary sequences, while a quencher is attached to the other.

If the amplicon contains the desired target sequence the probe hybridizes to complementary strands of DNA in the amplicon. In the new configuration where the probe has hybridized the fluorescein produces fluorescence as it would have been separated from the quencher. However, in cases where the amplicon does not contain desired target sequence the probe does not hybridize to the amplicon. In this case it is the complementary sequences that flank the probe that will remain hybridized to each other. Also, fluorescence is not produced because the quencher and the fluorescein will be in close contact (Farrell 2017:309–310).

2.7.6.2 Application of molecular beacon technology in the GeneXpert

In the GeneXpert molecular beacon technology is employed to detect the presence of 5 sequences from the RRDR. The detection of MTB and rifampicin resistance is based on the measurement of cycle threshold (C_T) for each of the 5 probes. Wanger et al (2017:247) define CT as the cycle number at which the fluorescence generated by the products of PCR crosses the fluorescence threshold and is considered positive. The growth of copies of target DNA produced during PCR is exponential thus there is a relationship between CT and the initial amount of target DNA that was in the specimen. A specimen containing many copies of target DNA to begin with will have a lower CT value while one that has low amounts will have a high CT.

In the GeneXpert the relationship between CT and amount of MTB DNA in the specimen has been extrapolated to produce a semi quantitative scale for the amount of MTB detected in the specimen.

2.7.6.3 Detection of mycobacterium tuberculosis

Lawn and Nicol (2012:[4]) explain how the GeneXpert applies the concept of CT to both the detection of MTB and rifampicin resistance. The CT of the first probe to be positive is termed the early CT while that of the last probe to be detected is called the late CT. A sample is positive for MTB if any two out of the five probes are detected within 2 cycles of each other. This means that the difference between the first and second CT values should be less or equal to 2. By contrast other molecular rapid molecular methods base their identification of MTB on probing for the presence of MTB specific sequences such as the insertion sequence IS6110. At the same time the relationship between CT and the amount of target DNA in the sample is applied to the early CT to give a semi-quantitative interpretation of how much MTB was in the sample. According to the GeneXpert package insert the instrument software will interpret CT values as shown in Table 2.1.

Table 2.1 How GeneXpert software interprets Ct Values

Value of early CT	MTB Result
CT ≤ 16	MTB detected, High
CT >16 – ≤22 ≤	MTB detected, Medium
CT >22 – ≤28	MTB detected, Low
CT >28 – < 38	MTB detected, Very Low

(GeneXpert MTB 2013)

2.7.6.4 Detection of rifampicin resistance

Rifampicin resistance detection is determined based on the difference between the values of the early and late CTs also called ΔCT. According to the Blakemore et al (2011:2496), a strain is considered resistant if the ΔCT is greater than 4. This differs from what is described by Lawn and Nicol (2012:[4]) who place the ΔCT for the GeneXpert software to recognize resistance at 3.5. This is because in 2010 Cepheid upgraded to the GeneXpert cartridge from version 3 to 4. A brief description of the changes included in version 4 and its performance characteristics is included in section 2.8.5.5.

The rationale for using ΔCT for determining rifampicin resistance is that when there are no mutations, the target DNA amplicon has a DNA sequence similar to that of wild type mycobacterium tuberculosis thus GeneXpert probes will hybridize to the product with ease and almost at the same time. However, when there are mutations in the RRDR, they inhibit the hybridization of probe and target DNA amplicon. In some cases, a mutation causes a reduction in the signal from the corresponding probe so that the probe is detected later than the others while in some cases the signal is eliminated completely. Blakemore et al (2010:2496) aptly termed the earlier as delays and the later as dropouts.

2.7.6.5 GeneXpert MTB/Rif version 4 and its performance characteristics

According to a 2011 report by the Foundation for Innovative New Diagnostics (FIND [2011]), the new version was meant to correct for test failures and optimize detection of rifampicin resistance. The main changes included an adjustment to the fluidics to eliminate errors, an update of the assay settings, reduction in annealing time for PCR2,

modification of the sequence of probe B and the addition of a fluorescent tracer for the probe check control.

FIND (2011) reports that between the GeneXpert versions 3 and 4, there was no statistically significant difference in the detection of TB and rifampicin resistance in sputum whether culture positive or culture negative. There was however a statistically significant reduction in the proportion of errors in general and specifically errors of code 5011 in version 4 as compared to version 3. Osman, Simpson, Caldwell, Bosman and Nicol (2014:[636]) on the other hand point out that version 4 has a higher positive predictive value for rifampicin resistance than the version 3. Their findings suggest that version is specific for rifampicin resistance detection compared to version 3. It is not clear why FIND did not detect an increase in specificity for rifampicin resistance as did Osman et al (2014:[636]). A possible explanation may lie in the fact that FIND only report on a small number (12), of rifampicin resistant strains from three geographical locations including South Africa, Uganda and Azerbaijan. The small sample may not have enough variability in the mutations coding for rifampicin so that the mutations are detected well by both versions. On the other hand, Osman et al (2014:[636]) report on 196 rifampicin resistant strains from the Western Cape in south Africa thus it is plausible that there was a wider variability in the mutation coding for rifampicin resistance to include those where version 4 was better at detecting. Besides the findings of Osman et al (2014:[636]), are plausible given that optimizing rifampicin resistance detection is one of the stated aims of the revision from GeneXpert version 3 to 4. In any case for the purpose of this study only GeneXpert version 4 matters because for the period covered by the study (2012–2016), study sites were using version 4 of the GeneXpert.

Having looked at how the GeneXpert works and generates its data this review moves to focus on the applications of GeneXpert. The application of GeneXpert for the accurate earlier diagnosis of TB and rifampicin resistance is its most widespread use. Other purposes of the GeneXpert which are reviewed here include; use of GeneXpert data for infection control purposes, for molecular epidemiology and use of reaction products from the GeneXpert to feed other molecular tests. The main argument of this review is that the gaps that exists in the exploitation of GeneXpert data for molecular epidemiology relate to scale and systematic use.

2.8 WHO'S GENEXPERT POLICY OF 2010

The use of GeneXpert for detection of MTB and rifampicin resistance is supported by two policy frameworks. The first was developed 2010 and the second one which was an update to the first one, was developed in 2013. Even though the second policy on the use of GeneXpert essentially replaces the first, both are reviewed below.

2.8.1 Evidence base for 2010 GeneXpert policy

The WHO convened a group of international experts in September 2010 to review evidence on the performance of and make recommendations on the GeneXpert. The group was herein after referred to as the expert group of 2010 and its members are listed in annexure G. The evidence base for the recommendation developed in 2010 is comprised of findings from three sources namely analytical, clinical validation and field demonstration studies.

2.8.2 Evidence from analytical studies

The works of Banada et al (2010), Blackmore et al (2010) and Helb, Jones, Story, Boehme, Wallace, Ho, Kop, Owens, Rodgers, Banada, Safi, Blakemore, Lan, Jones-Lopez, Levi, Burday, Ayakaka, Mugerwa, McMillan, Winn-Deen, Christel, Dailey, Perkins, Persing, and Alland (2010) constitute are part of the analytical studies which contributed evidence that related to the sensitivity of GeneXpert, specifically in the dimension of limits of detection and aspects on biosafety. Helb et al (2010) determined that the limit of detection for the GeneXpert is 5 genome copies which is equivalent to 131 colony forming units per millimetre of sputum. In contrast Toman (2004:11) explains that for smear to be positive with a grade of 'scanty' requires at least 5000 bacilli per millimetre. As well, Dorman (2011) testifies to the poor sensitivity of microscopy when she states that its limit of detection is approximately 10 000 colony forming units per millimetre. Therefore, the sensitivity of GeneXpert is far superior to that of microscopy. The Expert group of 2010 also established that GeneXpert had a specificity of >99.5% for detecting mutations associated with rifampicin resistance in the RRDR.

Regarding the safety of operating the GeneXpert, Banada et al (2010:3552–3555) showed that no infectious aerosols were produced during both the hands-on and in–

machine processing phases of the GeneXpert. This was so even, when samples were spiked with high bacillary loads of mycobacteria from culture. Helb et al (2010:[232]) complement these findings by establishing that the GeneXpert sample reagent makes a log – 8 killing of bacilli present in the sample in the given fifteen minutes reaction time. This is still in agreement with the researcher's observation that biosafety studies on the GeneXpert refer to events after the sample preparation by the operator are done. But the shaking of the sample is done two times both of which are before the points where studies describe.

The last aspect demonstrated by analytical studies was the specificity of GeneXpert for detection of MTB. Specificity in this case concerns the ability of GeneXpert to discriminate MTB from non-MTB species in negative specimens spiked with non-tuberculous mycobacteria, fungi or viruses. The GeneXpert was able to correctly return results of "MTB NOT DETECTED" in all instances of non-tuberculous mycobacteria, fungi or viruses (Helb et al 2010:[233]).

2.8.3 Evidence from clinical validation studies

Boehme, Nabeta, Hillemann, Nicol, Shenai, Krapp, Allen, Tahirli, Blakemore, Rustomjee, Milovic, Jones, O'Brien, Persing, Ruesch-Gerdes, Gotuzzo, Rodrigues, Alland and Perkins (2010) and Helb et al (2010) carried out multi-centre clinical validation studies in epidemiologically diverse locations namely south Africa, Peru, Azerbaijan and India; and Vietnam and Uganda respectively. These were reviewed by the 2010 expert group in preparation for the development of the GeneXpert policy of 2010. The type of evidence contributed by these clinical validation studies focused on sensitivity and specificity of GeneXpert for detection MTB and Rifampicin resistance compared to culture and DST.

2.8.3.1 Performance of GeneXpert for detection of pulmonary tuberculosis

Boehme et al (2010:[1010]) determined that a single GeneXpert test has an overall sensitivity of 92.2% among culture positive samples whereas two tests would slightly increase the overall sensitivity to 96%. The comparison of sensitivity one test versus two is important considering that GeneXpert would in some cases replace smear microscopy as a first diagnostic test. In 2007, WHO had recommended that a diagnosis of TB be based on the examination of two smears (WHO 2007). Thus, the comparison of one

versus two tests sought to determine whether diagnosis of TB using GeneXpert should also necessarily require two tests.

Boehme et al (2010:[1012, 1013]) also showed that smear status of the patient affects sensitivity of the GeneXpert. They found that a single GeneXpert test has a sensitivity of 98.2% among smear positive – culture positive patients versus 72.5% among smear negative – culture positive ones. In this same vein the expert group of 2010 conclude that a second GeneXpert test increases sensitivity more significantly among smear negative – culture positive patients, i.e. from 72.5% to 85.1%. A third GeneXpert test among the same group of patients was also shown to increase sensitivity by 5.1%. Thus, in practice there is hardly need to request a third GeneXpert test.

The findings of Helb et al (2010:[233]) support those of Boehme et al (2010:[1012, 1013]), because they determined that in samples from Vietnam, GeneXpert had a sensitivity of 100% among smear positive – culture positive samples versus 84.6% among smear negative – culture positive ones. However, it should be noted that the clinical validation study by Helb et al (2010) involved 107 patients which is much smaller than the 1730 in the study of Boehme et al (2010).

Boehme et al (2010:[1010]) also found that GeneXpert sensitivity among patients infected with HIV was sufficiently high, at 93.9% compared with 98.4% among patients who are HIV negative. Patients infected with HIV are an important subgroup of TB patients because it has long been recognized that detecting TB in them using microscopy was difficult. While microscopy has lower sensitivity for detecting TB among HIV positive patients, a significant proportion of new TB patients globally are HIV positive. For instance, according to WHO 9% of new TB patients in 2017 were also infected with HIV. The situation is even worse in Africa where the WHO reports that 72% of new TB patients were infected with HIV (WHO 2018a:27). Therefore, the relatively high sensitivity of GeneXpert even among HIV infected patients makes it a viable solution for the problem of TB detection in these patients.

Boehme et al (2010:[1013]) also found that GeneXpert has a very low sensitivity among patients who are smear negative – culture negative. However, this is not surprising first because it is difficult to diagnose TB in this group of patients and second, that methods of TB diagnosis other than biological confirmation may in themselves be non-specific.

Both Boehme et al (2010) and Helb et al (2010) found that the GeneXpert had high specificity for detection of MTB. Consequently, the expert group of 2010 conclude that the overall specificity of GeneXpert is 99% when determined compare against microbiological methods plus clinical response of patients (WHO 2011:6).

2.8.3.2 Performance of GeneXpert for detection of Rifampicin resistance

GeneXpert was shown to have an overall sensitivity of 97.6% for the detection of rifampicin when phenotypic DST was used as the gold standard. The specificity was 100% (WHO 2011:6).

2.8.4 Evidence from demonstration studies

The expert group of 2010 also reviewed evidence from demonstration studies. As Boehme, Nicol, Nabeta, Michael, Gotuzzo, Tahirli, Gler, Blakemore, Worodria, Gray, Huang, Caceres, Mehdiyev, Raymond, Whitelaw, Sagadevan, Alexander, Albert, Cobelens, Cox, Alland and Perkins (2011) explain, the point of demonstration studies is to test the sensitivity and specificity of GeneXpert under conditions that closely resemble those in routine settings. The demonstration studies reviewed by the 2010 expert group were carried out in epidemiologically diverse locations including Uganda, South Africa, the Philippines, Azerbaijan, Peru and India.

It should be mentioned that the goal was to approximate routine conditions. Lawn and Nicol (2012:[10]) recommended that the routine conditions under which the performance of GeneXpert should be further tested should include district or subdistrict level of the health care system. As Steingart, Schiller, Horne, Pai, Boehme and Dendukuri (2014:[12]) suggest, GeneXpert is likely to have more impact if it is placed at levels of the health care systems closest to where patients receive their treatment. However, Boehme et al (2011) report about demonstrations studies conducted at peri urban primary health care centres in South Africa, MDR TB screening facilities in Azerbaijan and the Philippines and at a central hospital in Uganda. While peri urban primary health care centres would meet the suggestions of Lawn and Nicol (2012), it is not clear how far the MDR screening facilities in Azerbaijan and the Philippines fulfil this criterion.

In addition, the studies included only adults aged 18 and above who managed to give at least two sputum specimens within one week of each other. But in routine practice, the GeneXpert is used to diagnose TB from all patients suspected of TB including children. Thus, even in the demonstration studies, researchers maintained a degree of ideal conditions. Therefore, national TB programs wishing to decentralize the GeneXpert beyond the levels demonstrated here may experience performance different from the one the authors describe.

Boehme et al (2010:[1500]) find that from demonstration sites GeneXpert has a sensitivity ranging from 83.4% to 100.0% among all culture positive patients whether smear positive or negative. They concluded that the combined average sensitivity among all culture is 90.3%. As in clinical validation studies, GeneXpert has a differential sensitivity between smear positive and smear negative patients. That is, among smear positive patients the sensitivity of GeneXpert is 98.3% (ranging from 91.4%–100.0%) versus 76.9% (ranging from 56.3%–100.0%) among smear negative patients. On the other hand, Boehme et al (2010) found that GeneXpert had high specificity across study sites, overall 99.0% (range 97.7%–100.0%).

Lastly demonstration studies also show that mono rifampicin is very rare. This is also recognized by other authors such as Palomino and Martin (2014:[318]). Further, it confirms that rifampicin resistance is a good proxy for MDR TB. For the present study this is also significant because by extension our findings can also inform the national program not just about the epidemiology of rifampicin resistance but also that of multi-drug resistant TB.

2.8.5 Policy recommendations 2010

Based on the review of analytical studies, clinical trial studies and field demonstration studies WHO made recommendations in BOX 1.

1. Xpert MTB/RIF should be used as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB (strong recommendation).
2. Xpert MTB/RIF may be considered as a follow-on test to microscopy in settings where MDR-TB or HIV is of lesser concern, especially in further testing of smear-negative specimens (conditional recommendation acknowledging major resource implications).

Figure 2.2 Policy recomendations of the 2010 expert group

(WHO 2011)

2.9 GENEXPERT POLICY UPDATE OF 2013

The guidelines of 2010 addressed the diagnosis of TB in individuals in whom TB could be difficult to detect such as those infected with HIV and those in who smear microscopy was negative (WHO 2011). As well, the guidelines covered diagnosis of TB in those at high risk of having MDR TB. The clear gaps included detection of TB in children and patients with extrapulmonary TB. According to the WHO, in 2017 paediatric and extrapulmonary TB accounted for 10% and 14% of incident cases respectively (WHO 2018a:1, 82). Therefore, the 2013 GeneXpert policy update is an important document to review as it exhaustively addresses the three use cases of GeneXpert. These are diagnosis of TB and rifampicin resistance in adults, diagnosis of extrapulmonary TB and Diagnosis of TB in children.

2.9.1 Comparing 2010 and 2013 policies on GeneXpert

In May 2013 the WHO convened a group of experts to review available evidence and update recommendations on the use of GeneXpert. These experts were herein after referred to as the 2013 expert group and their full list is attached in annexure H. The 2013 expert group states that the purpose of the 2013 update is to replace the 2010 policy considering research subsequent to the writing of the 2010 policy (WHO 2013a:8). Above, the differences in how exhaustively cases in need of TB diagnosis are covered have already been discussed. Another source of the differences is in the questions that the respective reviews of 2010 and 2013 sought to answer.

The 2010 expert group sought to know the overall accuracy of GeneXpert plus its accuracy in smear negative samples. Alternatively, the 2013 expert group distinguishes 3 populations in whom they seek to establish accuracy of GeneXpert compared to either culture as a reference standard or a composite reference standard. These are adults, non-respiratory samples and children.

For adults the 2013 expert sought to determine the accuracy of different GeneXpert interventions that include:

i. GeneXpert used as replacement of smear microscopy.
ii. GeneXpert used as follow on test for smear negative patients.
iii. GeneXpert used for detection of TB in smear positive patients
iv. GeneXpert used for detection of TB in smear negative culture positive patients
v. GeneXpert used for the detection of TB in HIV positive patients
vi. GeneXpert used for the detection of TB in HIV negative patients
vii. GeneXpert used for detection of rifampicin resistance as a replacement of phenotypic DST (WHO 2013a:4).

Non-respiratory samples constitute the second population about which the expert group seeks to make recommendations. By contrast the 2010 policy had not addressed any questions to do with diagnosis of TB in non-respiratory samples. Perhaps because Cepheid had validated the GeneXpert only for sputum (FDA [s.a.]), plus at the time there may have been only a few research papers into the subject.

For non-respiratory the 2013 expert group sought to know the accuracy of the following GeneXpert interventions:

i. GeneXpert used to detect TB in non-respiratory samples in general as a replacement of usual practice when culture is the reference standard.
ii. GeneXpert used to diagnose TB in specific non-respiratory samples as a replacement of usual practice compared to a composite reference standard. The specific samples in question are lymph node, pleural fluid, cerebrospinal fluid, gastric fluid and tissue samples.
iii. GeneXpert used to detect rifampicin resistance in non-respiratory samples as replacement of phenotypic DST (WHO 2011:4).

The last target population distinguished by the 2013 expert group is children. The 2010 policy had also not made any pronouncement on GeneXpert for children. As in the other two populations the expert group seeks to know the accuracy of GeneXpert in interventions configured as follows:

i. GeneXpert used for detection of TB in children in general when culture is used as the reference standard.
ii. GeneXpert used for the detection of TB in children when a combined clinical and laboratory reference standard is used.
iii. GeneXpert used as a follow-on test in smear negative paediatric patients
iv. GeneXpert used for the diagnosis of TB when the comparative is microscopy.
v. GeneXpert for the detection rifampicin resistance in children as a replacement for usual practice (WHO 2013a:5).

2.9.2 Accuracy of GeneXpert for detection of tuberculosis and rifampicin resistance in adults

Steingart, Schiller, Horne, Pai, Boehme and Dendukuri (2014) conducted one the of the three systematic reviews on which the 2013 GeneXpert policy is based. Steingart et al (2014) are quoted here because their study is an update of the one that was used at the WHO meeting in May 2013, where the GeneXpert was updated. Thus, the 2014 review reflects the state-of-the-art evidence on the performance of GeneXpert for detection of MTB and rifampicin resistance in adults.

Steingart et al (2014:[15, 16]) estimate that when used as a first test for detecting MTB in adults, GeneXpert has sensitivity and specificity of 89% and 99% respectively. Further, the sensitivity and sensitivity of GeneXpert is 67% and 99% respectively, when used as add on test following negative smear microscopy. The findings of Steingart et al (2014:[15, 16]) complement what was already known about the performance of GeneXpert for detection. For instance, the expert group of 2010 had concluded that the sensitivity of one GeneXpert test is 92.2%. Although the expert group of 2010 did not provide confidence intervals, the researcher expects if calculated they would be overlap with the confidence intervals for the sensitivity of a single GeneXpert obtained by Steingart et al (2014:15).

Steingart et al (2014:[23, 24]) showed that the sensitivity of GeneXpert for detection of TB is affected by smear status and to a small extent HIV status of patients. This is illustrated by the finding that among smear positive – culture positive cases GeneXpert achieves a sensitivity of 98% which plummets to 68% among smear negative – culture positive patients. This makes sense because in any case it is more difficult to diagnose TB in smear negative cases. Besides both GeneXpert and microscopy rely on the presence of bacilli in sputum for detection, except that the earlier requires a smaller number to be positive.

HIV infection on the other hand, causes a slight decrease in TB detection sensitivity for GeneXpert. As Steingart et al (2014:[24]) report, GeneXpert showed a sensitivity of 86% (95% CrI 76–92) among HIV negative patients, and 79% among patients who are HIV positive. It would be interesting to also evaluate the effect of the interaction between HIV and smear status although this was not the focus of the 2013 expert group.

About the sensitivity and specificity of GeneXpert for the detection of rifampicin resistance Steingart et al (2014:[25]) find that GeneXpert achieves a high sensitivity and specificity of 95% and 98% respectively for detecting rifampicin resistance. But this means that, in 5% of cases GeneXpert is not able to accurately determine rifampicin resistance status. In section 2.3.2.2, the contribution of mutations outside the RRDR to this 5% has already been discussed. Another factor worth noting is the imperfect nature of current routine gold standards for rifampicin resistance detection.

Van Deun, Aung, Bola, Lebeke, Hossain, De Rijk, Rigouts, Gumusboga, Torrea and de Jong (2013:[2634, 2635]) demosntrate that MGIT which is one of the gold standards misses rifampicin resistance coded by certain low-level mutations. Therefore, using such as a reference method for GeneXpert which theoretically picks any mutation in the RRDR, may unnecessarily depress estimates for both sensitivity and specificity.

2.9.3 Accuracy of GeneXpert for detection of tuberculosis and rifampicin resistance in non-respiratory samples

The 2013 expert group finds that GeneXpert performs well in the detection of TB in different extrapulmonary samples that include lymph node tissue, lymph node aspirate,

cerebrospinal fluid, gastric fluid and other tissues. However, GeneXpert performs very poorly for detecting TB in pleural fluid (WHO 2013a:18–22). Measures of the performance of GeneXpert in non-respiratory samples in the studies reviewed by the 2013 expert group used culture and an author defined composite reference standard as the comparative. The composite reference standard in this case refers to a combination nucleic acid amplification tests other than GeneXpert, histology, smear microscopy, biochemical tests, presenting signs and response to treatment with anti-TB drugs.

With respect to detection of MTB in in lymph node tissue and aspirates, the 2013 expert group determined that GeneXpert has a median pooled sensitivity and specificity of 84.9% and 92.5% respectively, when culture is used as the gold standard. The median pooled sensitivity decreases to 83.7% when a composite reference standard is used (WHO 2013a:18, WHO 2013c:38).

For cerebrospinal fluid GeneXpert achieves a median pooled sensitivity and specificity of 79.5% and 98.6% when the comparator is culture. The median pooled sensitivity plummets to 55.5% when a composite reference standard is used. The 2013 expert group also identified that one the factors that enhance diagnosis of TB in CSF is the use of a concertation step (WHO 2013a:19).

The other two non-respiratory samples where the 2013 expert group found GeneXpert performing well, are gastric fluid and other tissue samples. In these two sample types a comparison was only made for the sensitivity of GeneXpert in detecting TB against culture. For gastric fluid the median pooled sensitivity and specificity of GeneXpert in detecting TB is 83.8% and 98.1% respectively. Likewise, pooled sensitivity and specificity is 81.2% and 98.1% respectively for other tissue samples (WHO 2013a:21, 22).

On the other hand, the 2013 expert group finds that GeneXpert has a pooled median sensitivity and specificity of 43.7% and 98.1% respectively, for detection of TB in pleural fluid. This sensitivity falls to only 17% when a composite reference is used. The 2013 expert group explains this finding by remarking that in general pleural fluid is a not a good sample to use for diagnosis of TB. Instead, they recognize pleural biopsy as a better alternative to pleural fluid for the diagnosis of TB (WHO 2013a:19).

It would be interesting to investigate the effect of including a concentration step before pleural fluid is tested by GeneXpert. The concentration step has been shown to improve sensitivity of GeneXpert for TB detection in cerebrospinal fluid (WHO 2013c:42). It is worthy some effort to find out whether the same applies for a concentration step for pleural fluid. Such effort is warranted because although it is already known that pleural biopsy is a better sample for TB diagnosis, in routine settings such as primary health care centres in developing countries there may be limited opportunities to obtain pleural biopsy. In any case it may be simpler to implement a concertation step before GeneXpert testing as GeneXpert may already be placed in labs that already have equipment and conditions for concentrating pleural fluid.

2.9.4 Accuracy of GeneXpert for detection of tuberculosis and rifampicin resistance in children

The review of the performance characteristics of GeneXpert in children is important for two reasons. First, it is difficult to diagnose TB in children for reasons ranging from difficulties in obtaining the sample to the paucibacillary nature of TB infections in children. This is in spite of the fact that the fact that WHO estimates that children carry 10% of the TB disease burden (WHO 2018a:82).

When compared against culture as a reference standard the sensitivity of GeneXpert for detecting TB in children is 66%. This falls to 4% when a composite reference standard that includes clinical diagnosis is used. The performance of GeneXpert in children reflects the difficulty in making TB diagnosis in children (WHO 2013a:26). The 2013 expert group also showed that there are nuances to the sensitivity of GeneXpert for detecting TB in children. As seen among adults, GeneXpert has a higher sensitivity among children who are smear positive – culture positive than those who are smear negative culture negative. For instance, the 2013 expert group reports that GeneXpert has a pooled median sensitivity of 96% in sputum of smear positive children compared to 55% in sputum of smear negative. Likewise, in smear positive gastric lavage GeneXpert has a sensitivity of 95% while in smear negative gastric lavage it has 62% (WHO 2013c:63).

Another factor affecting the sensitivity of GeneXpert is the age group of children. When the 2013 expert group divided children aged 0–15 in to 0–4 and 5–15 they showed that the median pooled sensitivity of GeneXpert was 57% in the 0 – 4 age group but it shot up

to 83% among the 5–15-year-olds (WHO 2013c:64). Thus, in conclusion the GeneXpert presents better prospects of improved detection of TB in children but its sensitivity is influenced by sample type, smear status and age group of the child.

2.9.5 Policy recommendations of 2013

Recommendations on the use of GeneXpert for detecting pulmonary TB and rifampicin in adults and children

- Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in adults suspected of having MDR-TB or HIV-associated TB (strong recommendation, high-quality evidence).
- Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in children suspected of having MDR-TB or HIV-associated TB (strong recommendation, very low-quality evidence).
- Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in all adults suspected of having TB (conditional recommendation acknowledging resource implications, high-quality evidence).
- Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in all children suspected of having TB (conditional recommendation acknowledging resource implications, very low-quality evidence).
- Xpert MTB/RIF may be used as a follow-on test to microscopy in adults suspected of having TB but not at risk of MDR-TB or HIV-associated TB, especially when further testing of smear-negative specimens is necessary (conditional recommendation acknowledging resource implications, high-quality evidence).

Recommendations on the use of GeneXpert for detecting pulmonary TB and rifampicin in adults and children

- Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for CSF specimens from patients suspected of having TB meningitis (strong recommendation given the urgency for rapid diagnosis, very low-quality evidence).
- Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture or histopathology) for testing specific non-respiratory specimens (lymph nodes and other tissues) from patients suspected of having extrapulmonary TB (conditional recommendation, very low-quality evidence).

Figure 2.3 Policy recommendations on the use of GeneXpert for detection TB and rifampicin resistance

(WHO 2013a:xv)

2.10 NEW USES FOR GENEXPERT

The full utility of GeneXpert is probably still being explored. This section briefly summarizes three interesting use cases of GeneXpert, its by-products and data. These are the application of GeneXpert or determining discontinuation of infection control, the use of remnants from GeneXpert for further molecular testing and the use of GeneXpert data for molecular epidemiology.

2.10.1 Use of GeneXpert for infection control

In the United States there are guidelines for prevention of nosocomial transmission of TB which requires that a patient suspected of having TB is placed in airborne infection isolation (AII), until TB infection can be ruled out. Previously ruling out of TB infection was based on a negative smear result for three smears taken at least eight hours apart. At least one of the three specimens were supposed to be a morning sputum. However, in 2015 the FDA cleared GeneXpert as an alternative method for ruling out TB in patients to be discharged from airborne infection isolation (CDC 2015a:[193]; Poonawala, Leekha, Medina-Moreno, Filippell, Johnson, Redfield & Saharia 2018:591).

The main advantage of using GeneXpert for ruling out TB for patients in AII lies in the potential savings in the time patients spend in isolation. Chaisson, Roemer, Cantu, Haller, Millman, Cattamanchi and Davis (2014:[1357]) found that GeneXpert reduced the amount of unnecessary time spent in AII to 35 hours and 45 hours when one and two GeneXpert tests replaced miucroscopy for AII discharge decisions. By contrast, when microscopy was used the amount of time spent in AII was at least 67 hours.

For AII, GeneXpert also bears the advantage that it is more sensitive than microscopy so that a single GeneXpert can replace three microscpy tests. As shown by Luetkemeyer, Firnhaber, Kendall, Wu, Benator, Mazurek, Havlir, Grinsztejn and Alland (2015), 1 and 2 GeneXpert tests are always more sensitive and specific than than 3 smear microscopy tests. Further, a single GeneXpert test identifies 96.7% of all patients with smear positive culture positive TB. In addition GeneXpert has a higher negative predictive value compared to microscopy. For instance Poonawala et al (2018:592) find a single GeneXpert test to have a NPV of 98% whereas that of microscopy is only 20%.

While the above is direct use of the GeneXpert for infection control another effort to apply GeneXpert data for infection control involves the use of CT values to predict the smear status of a patient. While this has not be so succesful, protagonists seek to establish a correlation between CT values and the smear status of a patient. Such correlation is important in TB wards that wish to separate patients into smear negative and smear positive cubicles. However as it is also known that approximately 20% of TB transmission occurs from smear negative patients, the efforts would still miss out some nosocomial re-

infection facilities by only seperating into smear neagtive and smear positive (Campos, Rocha, Willers & Silva 2016:[1]).

Fradejas, Ontanon, Munoz-Gallego, Ramirez-Vela and Lopez-Roa (2018:[10]) find that a cut off CT for ruling in smear positivity of 21.1 would have an acceptable sensitivity of 90.5% but a low specicity of 61%. Their results differ from those of Theron, Pinto, Peter, Mishra, Mishra, van Zyl-Smit, Sharma and Dheda (2012:[385]) who found that a cut off value of 20.2 only had a sensitivity of 32% and a specificity of 97%. The differences in the results prompt earlier comments that efforts to correlate smear status and CT values have been less succesful. However they can also be explained by differences in sample sizes between the studies. Fradejas et al (2018) had a smaller sample size compared to that of Theron et al (2012). Perhaps another issue that keeps making it difficult to have a clear cut off is the different smear microscopy methods that are used. These different methods have different sensitivities and thus different cut off values.

2.10.2 Use of GeneXpert by-products for further molecular testing

Extremely Drug Resistant (XDR), TB refers to MDR which is additionally resistant to at least a fluroquinolone and an injectable anti-TB drug. XDR strains or those that resist either fluroquinolones or injectables are less likely to be treated successfully through the standard MDR or short course regimen. Thus, the WHO recommends that for strains detected to be rifampicin resistant by GeneXpert there is a need to carry out further drug sensitivity testing for second line drugs using either rapid molecular methods such MTBDRsl (Hain Life Science GmBH), or phenotypic DST (Gilpin, Korobitsyn & Weyer 2016:[146]).

At the moment this often means that clinicians must request for an additional specimen from the patient. However long delays can occur so that by the time a second sample is collected culture may need to be done first as the new sample may be lower on bacillary load compared to the initial one used for GeneXpert. Besides delays, other problems include the fact that higher level of BSL facilities are then required for the decontamination and extraction of DNA.

Three groups of authors Alame-Emane et al (2017); Venter, Derendinger, De Vos, Pillay, Dolby, Simpson, Kitchin, Ruiters, Van Helden, Warren and Theron (2017) and

Mambuque, Abascal, Venter, Bulo, Bouza, Theron, García-Basteiro and García-de-Viedma (2018) take advantage of the decontamination step of GeneXpert to perform further molecular testing without requiring additional samples or higher BSL infrastructure with fair success.

While Alame-Emane et al (2017) and Mambuque et al (2018) used remnants of liquified GeneXpert specimens, Venter et al (2017) employed cartridge extract to carry out their further molecular testing. Using remnants of liquified GeneXpert specimen means that the researchers would take the remaining fluid in the sample chamber of the GeneXpert cartridge. On the other hand, researchers who use the cartridge extract use a fine needle to pierce and withdraw about 15 uL from the PCR reaction tube of the cartridge. What all the three groups of authors demonstrated is that it is possible perform further molecular testing without having to do culture first. They also showed that some of the factors that affect the possibility to obtain meaningful results from GeneXpert by-products, include the duration of delay between GeneXpert testing and further molecular testing, bacillary load in the initial sample and conditions under which the sample is stored.

Their findings are also important for developing countries which bear a disproportionate TB disease burden, lack facilities for culture, but have widely implemented GeneXpert. For instance, this is the case in Gabon according to Alame-Emane et al (2017:[2106]). Thus, possibilities to carry out further molecular testing that includes spoligotyping and sequencing may finally mean that the gap is molecular epidemiology of TB in these countries can be covered. Some of the issues that may need to be addressed if representative molecular typing surveys secondary to GeneXpert were to be a success include addressing how to get complete results even from paucibacillary samples.

2.10.3 Use of GeneXpert data to predict mutations conferring resistance

A third innovative use of GeneXpert data is the use of deep GeneXpert machine data to predict the mutations conferring resistance. In the simplest form of this use case, researchers mine deep GeneXpert machine data. Then they analyse the proportions of rifampicin resistance and calculate the frequencies of each probe in which a mutation is found. This is the basic approach taken by authors Ullah, Shah, Basit, Ali, Khan, Ullah, Ihtesham, Mehreen, Mughal and Javaid (2016); Kanade, Nataraj, Mehta and Shah (2019); Reddy and Alvarez-Uria (2017) and Ochang, Udoh, Emanghe, Tiku, Offor, Odo,

Nkombe, Owuna, Obeten and Meremikwu (2016). However, these studies often differ in how comprehensively they are in terms of place and number of participants.

The study by Kanade et al (2019) is by far the most comprehensive in its geographical and temporal span. It covers Mumbai city and includes patients across a five-year period from 201 –2017. As a result, they report patterns of missing probes from 686 rifampicin resistant strains. Ullah et al (2016) are also comprehensive in that even though they cover a district in Pakistan, it is for the years 2011–2014 and they include 408 isolates.

The present study made use of GeneXpert data to describe proportions of rifampicin resistance for a sample of patients tested by GeneXpert at selected sites in Swaziland for the years 2012–2016. Further it described the patterns of probes in which mutations are located. This study applied principles of epidemiology in seeking to describe proportions of resistance according to person, place and time. Further while patterns of probes in which mutation lie have been described in India, Nigeria, Uganda and Pakistan, there is no study to the knowledge of the researcher that has described them in Swaziland.

2.11 SUMMARY

This chapter reviewed what is already known about how the GeneXpert works, the regulatory frameworks that facilitate its major use in detection of TB and rifampicin resistance plus new ways in which its by-products and data are being used. In terms of detection of TB, challenges still exist in the diagnosis of TB in children and pleural fluid. This is perhaps not so much as a result of the sensitivity of the GeneXpert per se but due physiological factors which limit the availability of bacilli in pleural fluid and specimens from children. Future efforts to improve diagnosis of TB in pleural fluid and children must probably focus on detection of mycobacterial secretion products.

The mining of GeneXpert data is an area being explored by more researchers. Possible uses include description of mutations in given populations. Several previous studies have described mutations in different geographical locations including South Africa, Nigeria, India, Pakistan and Uganda. Except for a few, most such studies have limited coverage in terms of time and geographical space. Even though WHO has added GeneXpert as an alternative method to be used for reporting rifampicin resistance, none of the studies reviewed include the suggested WHO reporting format. This format is whereby rifampicin

resistance proportions are reported disaggregated by age group, sex, TB treatment history and HIV status. The current study contributes to covering this gap by reporting proportions of rifampicin resistance according to GeneXpert for Swaziland.

CHAPTER 3

RESEARCH DESIGN AND METHODS

"Facts alone, no matter how numerous or verifiable, do not automatically arrange themselves into an intelligible, or truthful, picture of the world."

— Francis Bello

3.1 INTRODUCTION

The purpose of this chapter is to provide the reader with detailed descriptions of the research design. The chapter begins by defining what is meant by research design, identifying the relevant way in which research is classified in general and specifically for epidemiological studies. The chapter then describes the target population and sample followed by the methods used to collect and analyse data. The chapter finishes with a discussion on how ethical issues were handled for this study.

3.2 RESEARCH DESIGN

Research design refers to the basic methods that were used to gather evidence needed to answer research questions. While authors like Abbott and McKinney (2013:35) and Vogt, Gardener and Haeffele (2012:3) concur on the above idea of design, University of Southern California ([USC] (2019) broadens the concept of research design by including aspects of data measurement and analysis of data. Perhaps rightly so, as Morroni and Myer (2014:78) point out sampling and data analysis methods depend on the chosen research design.

Abbott and McKinney (2013:35) and Vogt et al (2012:3) again concur on the classifying of research designs by dividing them into two broad categories namely quantitative and qualitative designs. The salient features of quantitative designs include measurement of phenomena in numbers and description of large populations by use of statistics. Surveys and experiments are examples of quantitative research designs.

On the other hand, qualitative designs are characterised by describing phenomena in words (Skinner 2014:349). Through quality of description and detailed reporting of human processes qualitative research can contribute to in depth understanding of a given research problem. Field research and content analysis are examples of qualitative research designs.

According to Morron and Myer (2014:78), epidemiological research designs can be classified into observational and experimental designs. The design of the current study falls into the observational category, specifically the study follows a descriptive correlational design. This was an observational study because the researcher did not manipulate the subjects in any way. Instead the study describes patterns of rifampicin resistance according to GeneXpert in terms of time (years 2012–2016), place (four regions of Swaziland) and person (gender, age group). A correlational element exists in that the study also determines correlations between rifampicin resistance occurrence on the one hand and time, place and person on the other.

3.2.1 Strengths of the descriptive correlational design

According to Polit and Beck (2012:229), the descriptive correlational design will be used in health research for a long time because it applies to many research problems where it is impossible to manipulate the independent variable. Further, they state that this design is efficient at collecting large amounts of data about a research problem. Morroni and Myer (2014:83) complement Polit and Beck (2012:229), when they posit that the descriptive correlational study design is relatively economical and easy to conduct when compared to a prospective design. In addition, its analytical components enable the exploration of whether there are any relationships between relatively fixed risk factors such as age, gender or place of living and occurrence of rifampicin resistance. The identification of any such relationships may serve as a basis upon which further studies employing more rigorous designs suited to identifying causality may be proposed.

3.2.2 Limitations of the descriptive correlational design

In the English language, in one sense, the word ‘limitations’ implies the imposition of limits on something or someone with regards to how good they can get (OALD [s.a.]). When applied to research design, Pyrczak and Bruce (2017:73) define limitations as

weaknesses that potentially limit the validity of research findings. Certain limitations are inherent in a study design so that they are outside the control of the researcher.

For the descriptive correlational design, Polit and Beck (2012:228) state that this design is weaker than experiments and quasi-experiments in supporting causal inferences. They offer two reasons why this may be so. First, the participants that researchers who use descriptive correlational design recruit are self-selected. Self-selection introduces selection bias so that the independent variable may not be equally distributed between groups. The second reason, also offered by Polit and Beck (2012:228) relate to confounding where there are complex relationships between variables causing researchers to fail to pinpoint which of the relationships are causal.

Morron and Myer (2014:84) add that causal relationships may also be difficult to pinpoint because in this design both exposure and outcomes are measured at the same time thus making it difficult to determine whether exposure happened before outcome. To attribute the observed outcome to a given exposure requires that the exposure precede the outcome and this condition is hardly satisfied by the current design (Ehrlich 2014:17).

3.3 SETTING AND POPULATION OF THE STUDY

3.3.1 Setting

3.3.1.1 Geographical setting

Setting refers to the physical place where the data for the study was collected (Polit & Beck 2012:743). Here setting is described in terms of geography and TB burden. Swaziland is small landlocked country covering an area of 17 364 km^2. It is almost enclosed by South Africa but also borders with Mozambique to its North east. Swaziland is administratively divided into four regions namely Hhohho, Manzini, Lubombo and Shiselweni with population almost equally distributed across the regions. According to the UN (2017), the population of Swaziland was 1.09 M in 2017.

3.3.1.2 HIV/TB disease burden in Swaziland

In 2017 the TB incidence in Swaziland was reported to be 308 per 100 000 population of with an incidence of 213 per 100 000 population HIV TB co-infection (WHO 2018a:249). At these rates Swaziland is included in the TB/HIV High Burden Country (HBC) list which was developed by WHO in 2016 (WHO 2017b:17). The TB/HIV HBC list includes 30 countries of which 20 have the highest absolute numbers of TB/HIV co-infected patients and remaining 10 have the highest incidence of TB/HIV co-infection. Swaziland is included in the list because of being among the ten countries with the highest incidence of TB/HIV.

3.3.1.3 Study sites

GeneXpert was first introduced in Swaziland in 2011 and by the end of 2012 there were 18 instruments covering the country's four administrative regions. From each region two health facilities were purposively selected for inclusion into the study. Sites with higher workload were prioritized for selection because this study sought to maximize representativeness of the study sample. Higher workload meant that the facility had a wider catchment area, thus improved representativeness. This also explains why three sites were included from Manzini region. The additional site caters for inhabitants from a densely populated area who mainly work in factories. This population has rather unique socio-economic dynamics, thus is included in the interest of maximizing diversity.

However, for privacy, study sites are named using a code that includes the region and a number for each facility (F). According to this notation the study sites were herein after referred to as Hhohho F1, Hhohho F2, Lubombo F1, Lubombo F2, Manzini F1, Manzini F2, Manzini F3, Shiselweni F1 and Shiselweni F2.

3.3.1.4 GeneXpert penetration in Swaziland

Xpert MTB/Rif was first introduced in Swaziland in 2011 and by the end of 2012 there were 18 instruments covering the whole country.

3.3.2 Target study population

Population refers to the complete set of persons about whom a researcher intends to make conclusions (Babbie 2014:207). It is normally impractical to study every single member of the population; therefore, researchers normally study a subset of the population called the target study population. The target study population should be selected in such a way that inferences can be made about the population from the findings of studying the sample. Representativeness is the concept that captures the essence of this requirement. For a study population to be representative, its elements must have characteristics that vary in approximately the same way as the population from which they are selected (Babbie 2014:206).

For this study, the target study population were TB patients who were confirmed to have TB by GeneXpert testing in Swaziland. Concerning TB patients in Swaziland, it is known that the number of patients notified to the National TB program declined by about 50% during the study period from 7731 in 2012 to 3806 in 2016. There is no clear reason for this decline, but it should be noted that the Government of the Kingdom of Swaziland (GKoS) has exerted concerted efforts to fight both HIV and TB in the past decade. About HIV/AIDS in Swaziland, UNAIDS (2018) reports that by 2018, Swaziland was one of the six countries to have achieved UNAIDS 90–90–90 targets for ending the HIV threat by 2030. On the TB front Haumba, Dlamini, Calnan, Ghazaryan, Smith-Arthur, Preko and Ehrenkranz (2015:[104, 105]) note that from 2010 Swaziland witnessed a decreasing trend for notified cases. The said decrease was more pronounced among smear negative pulmonary TB and previously treated patients. Haumba et al (2015:[104, 105]) also point out that HIV incidence decreased in Swaziland from 6% in 1999 to 2.3% in 2015. Thus, the decreases in TB case notifications seen in Swaziland from 2010, including the period covered by this study were also due bold TB control efforts of the GKoS. Haumba et al (2015:[104, 105]) also point out that Swaziland was one of the first countries to implement GeneXpert. Further she also aggressively implemented collaborative TB/HIV activities as recommended by the WHO.

Notified cases include bacteriologically confirmed cases and other cases that are confirmed clinically or otherwise. Therefore, the population of interest for the study is approximately that described in the 2016 annual TB report of the NTCP as sputum smear positive (GKoS 2016). This is because this group of patients includes only

bacteriologically confirmed cases of TB. Across the study period the proportion of notified cases that fall in the SS+ category has been increasing from 37% in 2012 to 61% in 2016. The study seeks to describe the prevalence of rifampicin resistance among patients diagnosed of TB by GeneXpert, for the years 2012 – 2016.

3.3.3 Eligibility criteria

Eligibility criteria can be divided into inclusion and exclusion criteria Eligibility criteria refer statements and conditions that specify who should or should not be included in a study. Babbie (2014:156), opines that eligibility criteria must be driven by theoretical considerations. This is because for Babbie eligibility criteria are linked with construct validity in the sense that when the subjects of the study have more characteristics in common with the population to which findings will be generalized then construct validity is enhanced. For this study the inclusion and exclusion criteria are described below.

3.3.3.1 Inclusion criteria

GeneXpert records were included in the study if:

- They had positive results for MTB on GeneXpert and had valid results for rifampicin resistance. Valid results for rifampicin resistance are either ‘Rifampicin resistance detected’ or Rifampicin resistance not detected
- The had a date of testing between and included 1 January 2012 and 31 December 2016.
- Had been tested at one of the nine selected sites
- Had been tested from a specimen on which it was possible to detect pulmonary TB. Pulmonary TB can be detected in sputum, induced sputum, and gastric specimens.

3.3.3.2 Exclusion criteria

GeneXpert records were excluded from the study if:

- They had inconclusive and or negative results for MTB on GeneXpert. Inconclusive results refer to errors on GeneXpert.

- They had date of testing less than 01 January 2012 or greater than 31 Decemeber 2016.
- Had been tested from extrapulmonary samples that include lymph node aspirate, synovial fluid, cerebrospinal fluid and pus.

3.4 SAMPLING METHODS

Babbie (2014:197) defines sampling as the process through which observations for a study are selected into the sample. The sample is a subset of the population which is studied so that inferences can be made about the population from which the sample was drawn. Abbott and Mckinney (2013:105) distinguish two broad categories of sampling namely probability and non-probability sampling.

In probability sampling each member of the population has an equal chance of being included in the sample. Simple random sampling, systematic random sampling, proportional to population size are examples of random sampling variations. The advantage of random sampling is that the resulting sample is more likely to be representative. Being representative means that the sample elements have variation in characteristics that approximates that of the population. Abbott and Mckinney (2013:106) also state that random sampling makes the sample more amenable to probabilistic statistical analysis.

By contrast, non-probability sampling does not make use of probability theory for selecting observations. Of interest to this study is purposive sampling that was used in the first step of sampling. Polit and Beck (2012:275) point out that one of the key features of purposive sampling is that the researcher selects observations based on their knowledge of the population characteristics.

This study made use of a combination of non-probability and probability sampling. in two stages. Polit and Beck (2012:275) refer to this as multi-stage sampling whereby, in the first step large population units are selected, followed by individuals in the second stage. In this study laboratories were the larger units selected purposively in the first stage while individuals were selected in the second stage.

3.4.1 Stage1: Purposeful nonprobability sampling

Multi-stage sampling was applied to ensure representativeness. In the first stage 9 diagnostic sites located in the four administrative regions of Swaziland were purposefully selected based on workload. Sites with higher workload were prioritized because the higher the workload, the more the samples to be tested. Higher workload also meant that the site had a larger coverage area. Including sites with a higher coverage area indirectly contributes to representativeness of the sample. Two sites were selected from each of Shiselweni, Hhohho and Lubombo regions while three were selected from Manzini region to include one GeneXpert site which caters for inhabitants from a densely populated area who mainly work in factories.

3.4.2 Stage 2: Random sampling

The goal of sampling is for a researcher to select members from a population so that the description of the characteristics of those members estimate that of the population of which they are a part. Joubert and Katzenellenbogen (2014:99) assert that random sampling can achieve the selection of representative sample because all members of the population have an equal chance of being included in the sample. Babbie (2014:203) states that the first advantage of random sampling is that it eliminates bias from the researcher as he/ she does not choose who to include in the sample. Further using probability sampling makes the sample amenable to the rules of probability theory and analysis. Both advantages suited the needs of the current study.

Therefore, in the second stage, random sampling was used to select 50% of all GeneXpert positive samples available for the years 2012–2016. Sampling 50% of positive Xpert MTB Rif is oversampling which the researcher justified as a device to include a large enough sample of rifampicin resistant strains. The researcher extracted data directly from the GeneXpert instrument. The data was extracted from the GeneXpert in two formats namely a complete listing of tests done in each of the years and data for all test in comma separated values (csv) format. A detailed procedure for the data collection is given below.

3.5 DATA COLLECTION METHODS AND PROCEDURES

3.5.1 Procedure for obtaining complete listing of all positives

The procedure for obtaining a complete listing of all positive GeneXpert tests for a given site, was as follows. GeneXpert DX software was started by double clicking the GeneXpert DX icon on the GeneXpert computer's desktop. In the main menu <<View results>> was selected. A snapshot screen appeared listing for each test: the lab number, patient name, assay name, result, assay status and the date test started. The researcher selected all entries for each of the years 2012–2016 and copied the data by pressing Control + C on the keyboard. The copied data was pasted into a new excel sheet which was saved on an external hard disk and transferred to a secure folder on the researcher's computer. The file was named following the pattern: site_name_complete_listing_201X e.g. Hhohho_F1_Complete_listing_2012.xlsx.

3.5.2 Procedure for obtaining data in csv format

GeneXpert DX software was started by double clicking the GeneXpert DX icon on the GeneXpert computer's desktop. In the main menu <<View results>> was selected. Then the topmost result was double clicked to expand its view. In the bottom left section of the resulting window, <<Export>> was selected. Then 4000 tests were selected for export at a time. The resulting comma separated values files was used to find the probe data for each of the included samples. The files were named following the pattern 'site_name_csv_201x'. For sites that did more than 4000 tests in a certain year, the naming pattern also included a serial for the csv file.

3.5.3 Procedure for obtaining data from paper registers

A portable hand-held scanner was used to scan GeneXpert lab registers for each of the years 2012–2016. The pdf file created was named following the pattern: Site_Lab_Register_201x. Where more than one file was created for the same year a serial number was similarly included in the file naming pattern.

A complete listing of the positive GeneXpert tests for each year was obtained by applying a filter to display only the observations with positive results on the file

site_Complete_listing_201x.xlsx. The observations with positive results comprised the complete list that was used for randomly selecting the 50% sample. They were copied into a new excel tab within the same worksheet. In the new tab a column was inserted where serial numbers were assigned to each of the positive observations. Microsoft excel was used to sample 50% of the Xpert MTB Rif positive samples. For the included samples, demographic data was obtained from the scans of the lab register and probe data was obtained from the file site_csv_201x.xlsx.

3.6 METHODOLOGICAL RIGOR

According to Marquart (2017:[1]), methodological rigor refers to the thoroughness and accuracy with which a study is conducted, of which reliability and validity are the two more prominent dimensions. Reliability of a measure implies that if repeated measurements are made using the same tool, similar results should be obtained if the measure is valid (Babbie 2014:152).

On the other hand, Polit and Beck (2012:236), describe validity as referring to the approximate truth of a measure. Meaning that, validity is the degree to which a measure reflects what it purports to be measuring. Moreover, it is linked both research design and inference. The way they are linked is that the research design determines what inferences can be made and validity is a property of inference. For example, if one carries out a case control study, they cannot validly claim to make inferences about incidence of the condition under study because the statistics possible to generate from a case control study include odds ratio and risk ratio. Consequently, Polit and Beck (2012:236) distinguish four types of validity namely statistical conclusion validity, construct validity, internal validity and external validity.

3.6.1 Statistical conclusion validity

Polit and Beck (2012:236, 237) say that statistical conclusion validity asks whether the relationships seen between variables are real or whether if they exist there sufficient power within the study to detect them. They also identify that statistical relationships may be masked in studies with low power, and in studies in which the intervention treatment was unfaithfully implemented. Thus in this study the strategy that was used to enhance statistical conclusion validity was having a large enough sample. In fact, instead of

calculating a sample size, the researcher justified and oversampled fifty percent of GeneXpert records from each year resulting in a sample with sufficient power to detect relationships.

3.6.2 Construct validity

Babbie (2014:156) defines construct validity as the extent to which a given measure relates to other variable in a system of theoretical relationships. On the other hand, Polit and Beck (2012:237), emphasize the validity of inferences observed in study to the constructs which the observed measures represent. Thus, in this study, construct validity would concern how far the proportion of rifampicin resistant GeneXpert records divided by the total of MTB positive GeneXpert records in a given period, represents the prevalence of rifampicin resistance. As the researcher employed well defined, previously used constructs, there was no need for additional strategies to enhance this study's construct validity. The construct of prevalence of rifampicin resistance was previously defined by the WHO (WHO 2015b:57).

3.6.3 Internal validity

Internal validity refers to the degree to which confounding can be ruled out when evaluating the relationships seen within a study. Naturally as this study was an observation study it was susceptible to a number of internal validity threats with fewer options available for the researcher to control. Statistical control was the most viable option available to this study. This was why relationships between age, gender and place were tested in two ways, first by Chi square test and after by logistic regression. Although the two tests are slightly different, a relationship demonstrated by both methods was considered to have higher internal validity.

3.6.4 External validity

As the term intuitively suggests external validity is about moving from inferences made within study to target populations. As Polit and Beck lso add generalizations may also need to be at individual patient level as in the case of evidence-based practice where clinician's concern is to apply treatments seen to effective within studies to individual patients. Babbie points out there is a tension between internal and external validity.

Enhancing one may result in attenuating the other. For instance, the more tightly a researcher controls who can be included in a study (thus enhancing internal validity), the less applicable to the general population which means external validity was attenuated. This study enhanced external validity by drawing GeneXpert records from multiple sites and randomly selecting which records would be included and analysed.

3.6.5 Research instrument testing and validation

In some social research fields like communication it is common for researcher to develop or adapt a study instrument which may be in the form of a questionnaire or observation plan. Marquart (2017:[2]) argues that such an instrument should be developed based on theoretical considerations and should be validated. The validation may involve pilot testing to determine usability, comprehension by both the research assistants administering the questionnaire and the participants. The end points of such testing may include reliability coefficients such Cohen's Kappa which measures inter-reader variability.

The missing of reporting about such testing for this study is based on the type of data this study dealt with. The data was mainly quantitative data being extracted from primary sources that included GeneXpert registers in paper and electronic form and GeneXpert deep machine data. The main requirement which the researcher ensured for the excel sheet that was used was to exhaustive capture information from both lab registers and deep machine data.

3.7 DATA MANAGEMENT AND ANALYSIS

3.7.1 Data management

Data management refers to the totality of procedures used to manage data before, during and after research (UNISA 2019). WHO (2015b:209) states that these procedures include data acquisition, handling, cleaning, analysis and archiving. As UNISA states there is a contemporary trend towards promoting and enabling the discovery and re-use of research data. Thus, some funders of researcher require a data management plan that spells out issues about the ownership of data and how it will be accessible for others.

3.7.1.1 Data acquisition

Data for this study was acquired from primary sources namely paper laboratory registers for GeneXpert, electronic laboratory registers for GeneXpert, the Laboratory Information System (LIS) and GeneXpert deep machine data. Data for Hhohho F2, Lubombo F1 was obtained only from paper registers. That for Hhohho F1, Lubombo F2 and Manzini F3 was obtained only from the LIS. Electronic laboratory registers were available for Manzini F1, Manzini F2, Shiselweni F1 and Shiselweni F2. GeneXpert deep machine data was available for Shiselweni F2, Manzini F1 and Manzini F2.

3.7.1.2 Database design, data entry and verification

Microsoft Excel (Office 365) was used to enter data for the research. To achieve a high quality of data, data validation was used in several fields to ensure that only the expected kind of data was entered in them. For instance, data validation rules ensured that only dates in the format mm/dd/yy were entered in the field date of test. Likewise, there were rules to ensure that only ‘m’, ‘f’ or ‘blank’ could be entered for gender, only ‘high’, ‘medium’, ‘low’ ‘very low’ or ‘blank’ could be entered for GeneXpert MTB result and only ‘detected’, ‘not detected’, ‘indeterminate’ or ‘blank’ could be entered for GeneXpert rifampicin resistance result. Furthermore, all GeneXpert positive observations were entered regardless of whether some of the data was missing or not.

The Microsoft Excel worksheet was password protected and stored on the researcher’s personal laptop. The researcher’s personal laptop was also password protected to prevent inadvertent unauthorized access to research data (Sheffield 2017). The scans of lab registers and data extracts from the GeneXpert comprise the source documents for this study. They were be stored on the researcher’s computer with a backup on the external hard disk for a minimum of five years after the publishing of the dissertation.

The researcher is aware that WHO (2015b:209) discourages the use of Microsoft Excel as a database. However, its use for this study is justified because the number of variables the study dealt with is small. Therefore, Microsoft Excel was still able to deal with them.

3.7.2 Analysis plan

3.7.2.1 Patient flow

A patient flow diagram was created to show the steps at which eligible patients were excluded from further analysis. Patients who were excluded include the 50% who were not included in the random sample, those selected in the random sample but had missing rifampicin resistance results and those selected in the random sample and had indeterminate results for rifampicin resistance. Then SAS studio version 9.04 maintenance 6 was used to for the statistical analyses below.

3.7.2.2 Enrolment analysis

Enrolment analysis was done to show how many patients were enrolled from each of the four regions of the Swaziland. Such analysis is useful to demonstrate degree of representativeness of the data.

3.7.2.3 Analysis of GeneXpert records demographic characteristics

The proportions of GeneXpert records by gender and age groups were calculated to show the demographic composition of the data set. The age groups used were 0–14, 15–24, 25–44, 45–54, 55–64, 65 and above, as suggested by the WHO (2015b:23). The age groups suggested by the WHO are a common framework which many studies and national TB control programs use to report their data. Thus, their use allows comparison of data between different studies and or countries.

Then the proportions of rifampicin resistance were determined for each of the years 2012–2016 to describe rifampicin resistance trends. Proportions of rifampicin resistance were also calculated for in each year by age group, region and gender. This was done to determine if there were any differences in the extent to which rifampicin resistance affects different genders, age groups and residents of different regions.

It had been planned to analyse the proportions of rifampicin resistance among new and previously treated patients. The analysis was not done because laboratory registers did not have information on treatment history. An alternative could have been to seek this

information from TB treatment registers. This option was not followed because patients often start treatment in facilities of their choice which may be many in number and far from the testing site. Sometimes even in a different region than where test was taken. In addition, there are GeneXpert positive patients who never started treatment. Thus, the researcher expected that attempting to get treatment history from treatment registers would have meant a lot of effort with no guarantee that the information would be complete. In other words, the costs of following the possibility of using treatment registers to complement the data outweighed the benefits.

3.7.2.4 Analysis of mechanisms of resistance

Frequencies of delays and dropout were also calculated in order to determine through which of the two mechanisms, rifampicin resistance more commonly manifests itself. Further this study calculated the frequencies of the probes A–E, in which the mutations were located.

3.8 ETHICAL ISSUES

3.8.1 Defining ethics

Ethics is associated with issues of morality so that in a given community there are standards of what is right or wrong. Singh, Kagee and Swartz (2014:33) seem to concur when they assert that ethics is variously defined around the themes of moral values that should guide conduct of persons or members of a professional group. In medical research ethical principles were prompted by historical experiments which caused harm to their participants.

An example of such research is the Tuskegee syphilis study conducted between 1932 and 1972. Researchers sought to describe the natural history of syphilis, so they followed up a group of 399 male cases of syphilis and 201 controls (CDC 2015b). Researchers caused harm to the participants because they wanted to follow the men until death so that even when penicillin became recognized as an effective treatment, they prevented participants from accessing it. A lapse in ethical conduct saw the researchers prioritize the pursuit of knowledge over acting in ways that were fair, just and beneficial to the participants (Babbie 2014:65).

For epidemiological research Singh, Kagee and Swartz (2014:36, 37) consider that researchers must be guided by the notions of informed consent, confidentiality and equitable distribution of burden. The first two have a bearing on this study and will be briefly described below.

3.8.2 Informed consent

Babbie (2014:68) defines informed consent as a practice where subjects of a study only participate after they have understood the objectives, procedures and possible risks of the study. Informed consent requires that the participants have the capacity to understand the information about the study and choose voluntarily to participate. As the current study makes use of data previously collected for routine diagnosis of TB, over a period of five years it was impractical to seek informed consent from each of the study participants. All the same, the researcher sought ethical clearance for carrying out the study from the Health Studies Research Ethics Committee (HSREC [REC 012714-039]) at the University of South Africa (Annexure A). In Swaziland ethical clearance was also sought from the National Health Research Review Board (NHRRB [Annexure B, D]). In addition, permission was also requested from the Chief technologist of the Swaziland Health Laboratory Services (SHLS), who is the custodian of the laboratory data and from the matron at each hospital where each of the participating laboratory sites were located (Annexure C).

3.8.3 Confidentiality and anonymity

Protecting the identity of study participants is one of the important ways of ensuring that study participants are not harmed as a result of taking part in a research study. Babbie (2014:69) distinguishes two levels of participant identity protection namely confidentiality and anonymity.

Anonymity is achieved when both the researcher and the readers have no knowledge of the identity of the participant (Babbie 2014:68). This may suit studies that collect sensitive information, but the disadvantage is that the researcher may not be able to follow up participants in case of non-response or need. On the other hand, confidentiality is the status of identity protection when the researcher knows the identity of the participant but

promises to not reveal it (Polit & Beck 2012:162). Since the current study makes use of routinely collected data which already comes with the identity of the participants, confidentiality is the most suitable way to protect the identity of this study's participants. Further following all data cleaning and verification procedures the researcher created an anonymized data set that was used for analysis.

3.8.3.1 Strategies used for confidentiality

The researcher attempted to achieve both confidentiality and anonymity during this study. Confidentiality was the privacy level achieved for raw patient data. Raw data which included photographs of paper registers, excel based laboratory registers and extracts from GeneXpert were kept in a password protected folder on the researcher's computer. This achieved confidentiality in the sense that although the researcher had access to patient details and results, he restricted access to the data and did not share patient details with any other parties.

3.8.3.2 Strategies used for anonymity

The researcher also attempted to achieve anonymity for datasets created for analysis as part of the study. Data from the primary sources was captured in a excel sheet. Once the data was verified and cleaned, the name and surname columns were deleted and the new excel sheet without names was used for analysis. This achieves anonymity because once the names were removed even the researcher could not identify to who each GeneXpert record belonged. The anonymized dataset was also shared with a data analyst and it was safe to do so as the privacy of the patients behind the records was protected.

3.8.4 Research approval

Ethical approval was obtained from the Health Studies Research Ethics Committee (HSREC) (Annexure A), Ref number REC 012714-039]), from UNISA, and from the NHRRB, in Swaziland (Annexure B). Informed consent was not sought from the individual patients because the study used data collected routinely for clinical and diagnostic purposes. No data was requested directly from patients for the study. In fact, as there is a gap of two years between the last patient recruited and the writing of this study, the

researcher expected that most of the patients had already completed their contact with TB services at least for the TB episode for which they were recruited into this study. This made it more impractical to get informed consent from each of the persons behind the GeneXpert records.

The researcher also considered that there was very little chance that persons from whom the GeneXpert records were obtained would be harmed by participating in the study. It is important to note first and foremost that this was an observational study where the researcher was not introducing any experimental medicines into participants. However, the concept of harm would not be complete without considering the psychological dimension whereby participants suffer psychological trauma after participating in a study. Babbie (2014:66) suggests that some sophisticated participants may locate themselves in the presentation of results of a study they participated in and feel characterized. As this study relied on routine data the researcher is aware that the laboratory results of each individual patient have already been shared with the patient. What the researcher did was to produce aggregate measures about groups of patients and not individual patients. Even if persons behind the GeneXpert records were to read the findings of this study and somehow identify themselves as belonging to certain groups it would not come as a surprise to them as they already know their results.

The researcher also notes that as this study was for purposes of writing a dissertation. Readership for a dissertation is limited, with the primary readers being the examiners. The researcher made an undertaking to share the findings with the ministry of health, but the readership remains limited. This is not the primary method of preventing harm to participants, but it contributes to the same.

3.9 SUMMARY

This chapter identified and provided a detailed description of the descriptive correlation study design. After making a brief description of the design's strengths and limitations the chapter then described the study's target population and the sample that was selected. This was followed by a description of procedures for sampling, data collection and analysis. The chapter finished by discussing how ethical issues were handled for the study. In Chapter 4 findings of the study are presented.

CHAPTER 4

ANALYSIS, PRESENTATION AND DESCRIPTION OF THE RESEARCH FINDINGS

4.1 INTRODUCTION

The previous chapter presented the research design and provided the reader with detailed descriptions of what was done. This chapter presents the findings of this study and the analysis thereof. The flow of observations is presented first which shows how GeneXpert records were recruited from the various facilities in respective regions for each year. This was followed by a description of the demographic characteristics of the sample. Then proportions rifampicin resistance were presented, first in relation to the sample of the year as a whole then after as disaggregated by place, gender and age group. Association between common risk factors are also presented.

4.1.1 Flowchart of enrolled GeneXpert records

A flowchart of enrolled patients has been included as a first step for the presentation of results so that the reader can visualize steps in which eligible patients were excluded from the analysis (see Figure 4.1). This helps the reader to make an informed independent assessment of possible sources of bias and the extent to which they may influence the findings presented herein.

A total of 11319 patients with a positive GeneXpert result were obtained from the records of the 9 chosen laboratories. A random sample of 50% of all observations was selected from the yearly data of each site resulting in a total of 5667 patients being included in the sample. This is slightly more than 50% of the total number of patients with a positive result and is accounted for as follows: A 50% sample was selected using the ‘select random sample’ task in SAS studio, from each yearly sample per site. Thus, yearly samples containing odd numbers of observation SAS rounded up to the sample size to the next integer. For example, if a given year had a total of 37 observation SAS would pick 19 observations as it is not possible to pick 18.5 observations. As a result, after joining the

corresponding yearly samples from the 9 sites the total would naturally be slightly more than the total available patients divided by two.

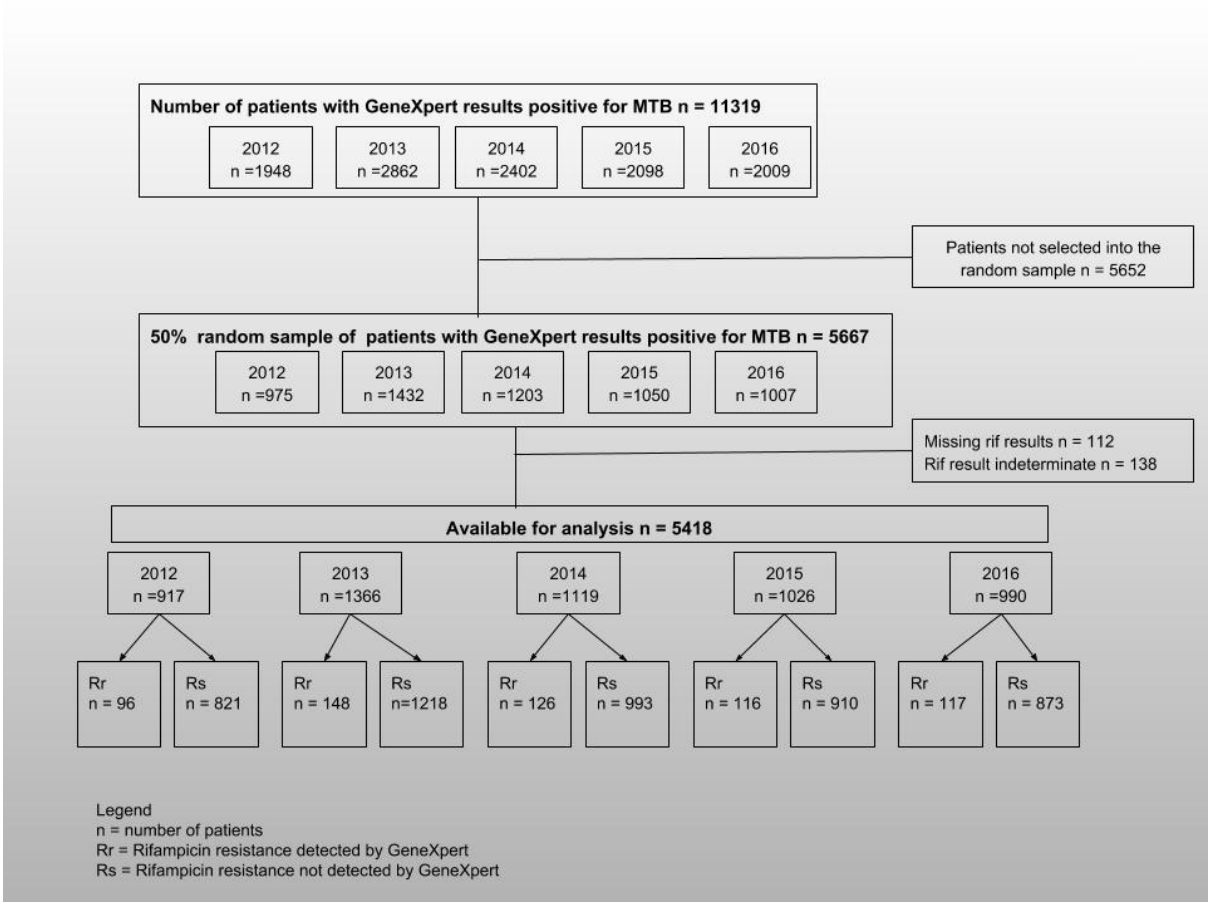


Figure 4.1 Flowchart of enrolled patients by year

4.1.2 Analysis of patient intake from sites

An analysis of intake of patients from sites was included here to assess the contributions of the various sites to each of the annual samples. Lubombo region contributed the lowest number of patients (n=570), while Manzini region contributed the most (n=1982). For Manzini this is mainly because 3 sites were included as opposed to two for the other regions. The relatively lower numbers from Lubombo region were partly because of missing lab records. In the case of Lubombo F1 records were missing for 2014, while in Lubombo F2 GeneXpert was only introduced in 2013. Records from Lubombo F2 for 2016 were not included as they were available only for 3 months.

The contribution to the sample by Shiselweni and Hhohho regions is comparable at 1431 and 1435 respectively. The data for patient intake disaggregated by facility and region is shown in Table 4.1.

Table 4.1 Analysis of patient intake by site

Region	Facility	2012	2013	2014	2015	2016	Total
Hhohho	Hhohho F1	112	263	240	210	195	1020
	Hhohho F2	49	113	105	90	58	415
Lubombo	Lubombo F1	87	153	0	42	126	408
	Lubombo F2	0	38	81	43	0	162
Manzini	Manzini F1	144	160	140	128	119	691
	Manzini F2	301	229	183	151	132	996
	Manzini F3	45	84	59	60	47	295
Shiselweni	Shiselweni F1	0	166	134	118	106	524
	Shiselweni F2	179	160	177	184	207	907
Total		**917**	**1366**	**1119**	**1026**	**990**	**5418**

4.1.3 Missing data

For clarity missing data in this study was discussed under three categories namely: missing laboratory records, laboratory records where the rifampicin resistance result was not recorded and cases where rifampicin resistance was indeterminate according to GeneXpert.

4.1.3.1 Missing laboratory records

Missing data refers to a situation whereby an observation has values on some variables but not on some. As Kang (2013:[402]) state, the problem of missing data is quite commonly experienced in research studies. Data is missing because of many reasons that include certain design features of surveys, refusal by respondents to answer questions that may be construed as intrusive or personal, missed appointments or disrupted clinical measurements.

A first step in dealing with missing data is to understand the structure of the missing data in relation to the whole dataset and specific variables. Berglund and Heeringa (2014:20, 23) distinguish two dimensions at which missing data structure can be classified. They are missing data pattern and missing data mechanism.

4.1.4 Missing data pattern

According to Berglund and Heeringa (2014:20), missing data pattern refers to the way in which missing variables are distributed in the data. The three examples of missing data patterns are arbitrary, monotone and missing by design. Berglund and Heeringa (2014:23) state that the arbitrary pattern may be the most common type whereby missing values are distributed in such a way that there is no pattern to the missing data structure. Monotone on the other hand is a systematic looking pattern which may be the result of data being collected in phases during a longitudinal study. Lastly missing by design is the third pattern which occurs in studies involving randomization which allows for missing data on specific variables on certain subsets of participants.

4.1.5 Missing data mechanisms

Missing data mechanisms differ from missing data patterns in that missing mechanisms apply to a single variable. A missing variable may be underlain by one of three mechanisms namely Missing Completely at Random (MCAR), Missing at Random (MAR) and Missing not at Random, (MNAR) (Berglund & Heeringa 2014:23).

4.1.5.1 Missing completely at random

Kang (2013:[2]) considers values as missing completely at random if the probability that a value is missing does not depend on other variables in the data set and does not depend on the value of that variable. MCAR is a stringent assumption to make and as Kang (2013) states some methods of dealing with data may not apply to MCAR.

4.1.5.2 Missing at random

MAR is a less stringent assumption on the missing observations on a given variable differ from the observed scores on that variable only by chance. The difference with MCAR is that the given variable may or may not depend on another variable in the model for MAR which is not permitted in MCAR.

4.1.5.3 Missing not at random

Values should be considered to be missing not at random if observations with missing data differ from those with complete data for some reason, rather than randomly. For example, is data from given region has missing values for some variable.

4.1.6 Methods of dealing with missing data

Gravelle (2013) elaborates three strategies of addressing missing values. They are list wise deletion, pairwise deletion and imputation.

4.1.6.1 Listwise deletion

Listwise deletion refers to the removal of all observations with any missing data. In the process significant amounts of original study data can be lost when listwise deletion is used. Table 4.2 shows the pattern of missing data in this study. If listwise deletion was used 6.7% (364/5054) would be lost. However, listwise deletion offers the advantage that it is simple to implement.

4.1.6.2 Pairwise deletion

Pairwise deletion means that calculations are made from all observations that have values present for the pair of variables involved (Gravelle 2013). For instance, when computing a 2 by 2 table of say gender by rifampicin resistance in SAS, the procedure 'proc freq' is used. It would include all observations where both gender and rifampicin resistance are present (5326) and delete the ones where either gender or rifampicin resistance is missing (92). Thus, compared to listwise deletion there would be a reduction in the proportion of data lost to 1.7%.

Pairwise deletion is also simple to implement but suffers from having different totals for different analyses (Gravelle 2013). If the example above is continued but this time to compute a table of age group by rifampicin resistance, similarly all pairs with values in both age group and rifampicin resistance would be included in the total. However, the total would be different from the one of gender by rifampicin resistance (5080 vs 5326). The differing totals may be confusing for readers.

4.1.6.3 Imputation

Imputation means refers to a collection of methods used to fill missing values with plausible values. The WHO (2015b:41, 42) recommends multiple imputation for dealing with missing survey data. Multiple imputation means that missing values are filled in several times the variability arising from imputation can thus be determined. In SAS proc MI is used to carry out multiple imputation.

Table 4.2 Item missing data pattern for 2012–2016

Group	Rifampicin resistance	Gender	Age group	Region	Frequency	Percent
1	X	X	X	X	5054	93.28
2	X	X	.	X	272	5.02
3	X	.	X	X	26	0.48
4	X	.	.	X	66	1.22

4.1.7 Handling of missing values in this study

In general, pairwise deletion was used in this study. It was selected because it is technically easy to implement. Further the proportion of missing data was small at a maximum of 6.2% for the table on distribution of rifampicin resistance by age group. Listwise deletion was used for logistic regression because it is the default mechanism for addressing missing values in SAS's proc logistic.

4.1.8 Distribution of missing laboratory records by site

The problem of missing laboratory records was experienced in three sites namely Lubombo F1, Lubombo F2 and Shiselweni F1. In Lubombo F1 the register for 2014 was completely missing while in Shiselweni F1 the registers for 2012 was missing. In both sites the missing records were paper based. Lubombo F2 on the other hand only had GeneXpert installed in 2013. However, data for Lubombo F2 for 2016 only consisted of 11 observations collected over 3 months thus this data was excluded because it may not be representative. In contrast with Shiselweni F1 and Lubombo F1, data from Lubombo F2 is stored electronically. Thus, the sample of 2012 excludes data from Shiselweni F1 and Lubombo F2, that of 2014 excludes records from Lubombo F1 while that of 2016

excludes data from Lubombo F2. Therefore, the samples of 2013 and 2015 includes data from all sites.

4.1.9 Missing Rifampicin resistance results

GeneXpert results consist of two parts; one part for the MTB detection and the other for the rifampicin resistance detection. During data collection from both electronic and paper registers there were cases where the result for MTB detection would be indicated but not the result of rifampicin resistance. Table 4.3 shows the distribution of missing rifampicin resistance results by facility. Manzini F2 and Manzini F3 had relatively more cases of missing rifampicin results. Combined with the observation that the highest total number of missing rifampicin results occurred in 2012, one can conclude that these clerical errors coincided with staff learning the new technologies. This may be true because GeneXpert was introduced in late 2011 and early 2012 for most facilities.

At the same time in Manzini F3 and Hhohho F1 a lab information system was also introduced around the same time. The advantages of the LIS can be somewhat seen from the pattern that the number of missing rifampicin resistance results decreased across the period for both Manzini F3 and Hhohho F1. On the other hand, Manzini F2, Shiselweni F1, Shiselweni F2, Manzini F1 and Hhohho F2 were using combinations of paper and Microsoft Excel. Essentially, they are manual result recording systems thus more difficult for the staff to maintain. Thus, while the errors of missing rifampicin results are fluctuating there is no discernible pattern.

Table 4.3 Distribution of missing rifampicin resistance results

	2012	2013	2014	2015	2016	Total
Shiselweni F1	-	4	6	-	4	14
Manzini F1	-	5	2	4	-	11
Manzini F2	5	11	1	4	3	24
Hhohho F1	9	3	1	-	1	14
Shiselweni F2	3	1	2	-	-	6
Hhohho F2	5	1	5	4	3	18
Manzini F3	15	-	9	-	1	25
Total	**37**	**25**	**26**	**12**	**12**	**112**

4.1.10 Rifampicin indeterminate results

In simple terms GeneXpert returns a result of rifampicin resistance indeterminate when the number of bacilli in the sample is very low. Thus, GeneXpert would have been able to determine that MTB is present in the test sample but is unable to determine presence or absence of mutations (Lawn & Nicol 2012:[4]). The researcher was faced with two choices which were either to include these patients in the denominator for calculating proportion of rifampicin resistance but not in the numerator or to exclude from analysis

Velasco (2010) views the idea of withdrawing from analysis, participants who have been included in a study to fall within the purview of exclusion criteria. According to Velasco (2010) exclusion criteria should be guided by the study objectives. In this s study a choice was made to exclude these patients with results of 'rifampicin resistance indeterminate' from analysis because this study was aimed at determining rifampicin resistance according to GeneXpert. Thus, data which lacked rifampicin resistance by GeneXpert is naturally not eligible. Moreover, the researcher could have included them but then run the risk of falsely depressing the proportions of rifampicin resistance in the sample. Lastly, traditionally drug sensitivity surveys included only smear positive samples. A sample with a result of 'MTB Detected, Very Low, Rifampicin resistance Indeterminate' most likely corresponds with a negative smear result (Theron et al 2012:[385]).

Table 4.4 shows the provenance of samples whose rifampicin result is indeterminate. From the data there seems to be a peak of indeterminate results in 2014 for most facilities.

Table 4.4 Distribution of indeterminate rifampicin resistance results

	2012	2013	2014	2015	2016	Total
Lubombo F1	5	7	-	2	-	14
Shiselweni F1	-	1	2	-	-	3
Manzini F1	2	6	11	3	-	22
Manzini F2	3	8	2-	1	2	34
Hhohho F1	1	8	13	2	3	27
Shiselweni F2	8	4	4	3	-	19
Hhohho F2	1	3	2	-	-	6
Lubombo F2	-	1	4	-	-	5
Manzini F3	1	3	2	2	-	8
Total	**21**	**41**	**58**	**13**	**5**	**138**

4.2 DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

4.2.1.1 Frequency of GeneXpert records by age

The rounded ages of persons from whom the GeneXpert records were obtained ranged from 1–99. In each of the years 2012–2016 most GeneXpert records belonged to the age group 25–44, both among females and males. Children as represented by the age group 0–14 and the elderly (in age group 65+), contributed lower numbers of GeneXpert records. Also, the male: female ratio for all persons from whom the GeneXpert records were obtained, with positive results for GeneXpert (MTB positive), is 1.07:1. However, this ratio is reversed for rifampicin resistance, wherein it is 0.86:1.

It is commonly held that lower numbers of children with TB could reflect the challenges associated with diagnosis of TB in children more so, biological confirmation (Brent, Mugo, Musyimi, Mutiso, Morpeth, Levin & Scott 2017:[1]). Without taking away from this explanation, it should be noted that traditionally very young children are often shielded from being in contact with many people. Coles (2017) suggests that some superstitious practices about babies have their roots in the need to shield infants from germs. In Swaziland, CATC ([s.a.]), narrate that traditionally, babies are kept away from men until they were older than 3 months. In addition, they were only allowed to play with other children when they are at least 3 years of age. These exclusionary practices may in part also explain why in very young children the incidence of TB may be comparatively lower than in the adult population. Besides with the coverage of BCG vaccination in Swaziland being over 90% one expects most young children to also benefit from the protection against infection afforded by vaccination (UNICEF 2017).

On the other hand, the lower numbers contributed by adults over 65 years of age are understandable because according to the demographic pyramid of Swaziland under 5% of people in Swaziland are age 65 and above in Swaziland (Index Mundi 2018).

4.2.1.2 Mean age of characteristics of GeneXpert records

Mean, mode and median are the three measures of central tendency. According to Polit and Beck (2012:384), measures of central tendency are important because they summarize data and show what are the typical values taken a by a given variable. In the

case of this study of this study measures of central tendency aid in answering the question of what the typical ages of persons from whom Gene with TB and rifampicin and rifampicin resistant TB were.

For a sample with *n* observations the mean is calculated by summing up all the scores and dividing by *n.* The mean is also known as the average or arithmetic mean. The mean is affected by extreme values and therefore it is most suitable for summarizing data which is symmetrical.

The mode is the most frequently occurring value in a distribution. The mode is more suited to summarizing nominal level data. However, Polit and Beck (2012:385) also point out that mode can fluctuate across several samples. The last measure of central tendency is the median which as the name suggests is the middle value, such that above and below it lies 50% of the scores.

The choice of which measure of central tendency to use in a study depends on the shape of the distribution of values. The shape of the distribution can be assessed by its kurtosis and skewness values. According to Abbott and McKinney (2013:379), kurtosis describes how peaked a distribution of values is. DiMaggio (2013:150) adds that kurtosis is also about the heaviness of tails of a distribution. A peaked distribution of values results when most of the values in the distribution cluster around the mean. According to DiMaggio (2013:150), a peaked distribution has a positive kurtosis value and is also called as platykurtic. On the other hand, flatter distribution with light tails has values spread away from the mean and a negative kurtosis value. DiMaggio (2013:150) also states that normality of the distribution can be assumed if the modulus of the kurtosis value is close to zero and not too far away from 1. In SAS, which was used for data analysis in this study, the shape of the data distribution was visualized by superimposing a kernel density plot on the histogram of distribution of age as shown in Figure 4.2.

Skewness describes the extent to which data tails off to the left or right of the mean in a distribution. Polit and Beck (2012:386) state that when a distribution is positively skewed if it tails off to the right whereas it would be negatively skewed if the distribution tailed off to the left. Further in a positively skewed distribution the mean is greater than the median and mode while the mean would be less than the median and mode in a negatively distribution.

Therefore, the shape of the distribution of values as described by skewness and kurtosis affects the choice of measure of central tendency to use because in very skewed distributions the mode and median may be more representative of the typical values of a variable than the mean. However, as Polit and Beck (2012:386) also mention, the choice of measure of central tendency to use also depends on the variable's level of measurement. Meaning to say that the mode is suitable when the variable being summarized is nominal, while the median applies for ordinal and interval variables and the mean applies for interval and ratio variables.

In this study, the mean was chosen as the measure of central tendency because the level of measurement for age is ratio. Also, as can be seen in Figure 4.2 the values of kurtosis and skewness were within the range -2 to +2 except for 2015 which had kurtosis and skewness values of 2.29 and 1.04 respectively. About the acceptable ranges for both kurtosis and skewness. George and Mallery (2016:114) state that values of greater than -2 and less than +2 are also acceptable for assuming normality of a distribution. Thus, in this study normality was assumed for data from each 2012–2016. Data from 2015 was also assumed to be normal given that while the kurtosis value for 2015 was just above 2, that of skewness was 1.04.

The mean ages were 34.2, 34.8, 35.7, 35.1 and 36.3 for the years 2012–2016 respectively. When disaggregated by gender, the mean age of males was slightly higher than that of females across all the years 2012–2016. Although not shown here, the results of *t* tests comparing the mean ages of males and females for each of the years had *p* values of <0.0001. Meaning that there is very little chance of observing these differences or greater, if there is no difference in the proportions of males and females.

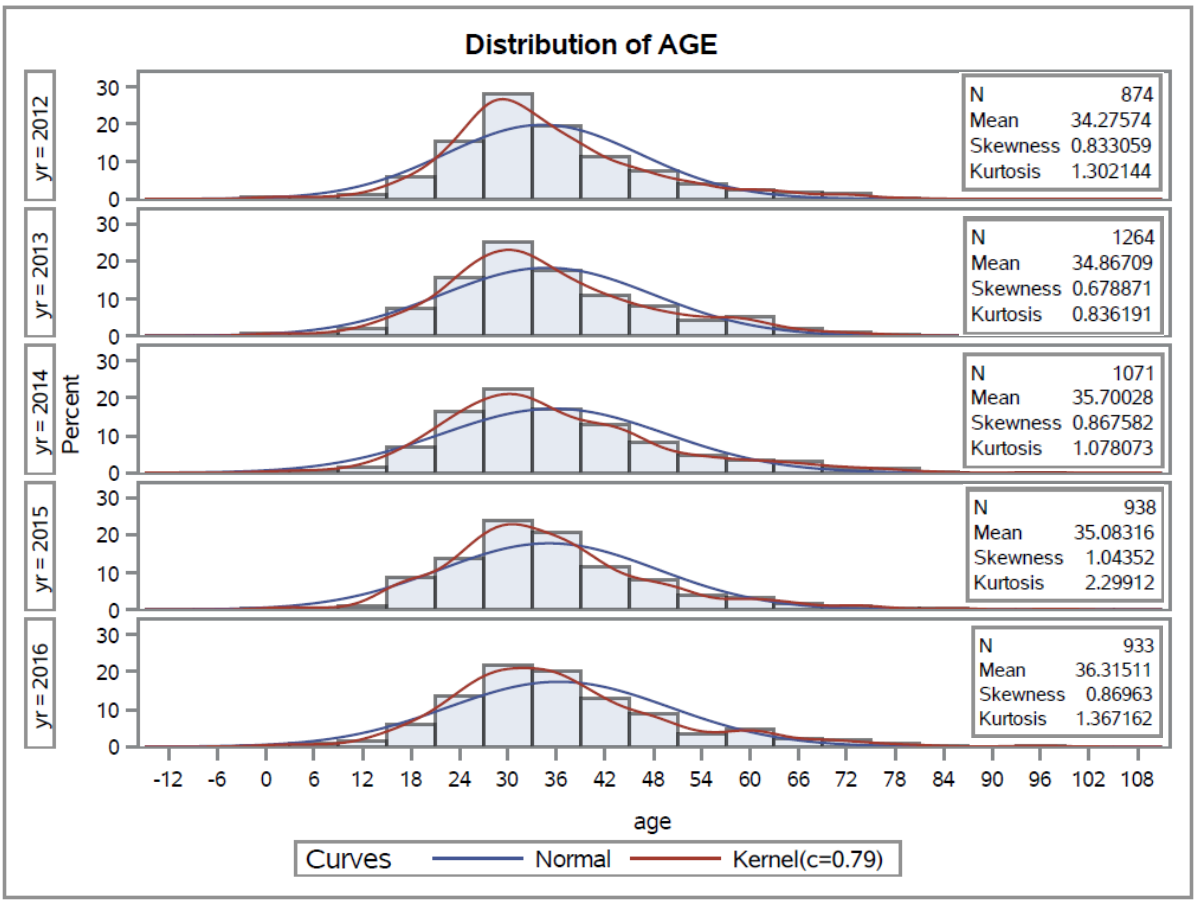


Figure 4.2 Shape of GeneXpert records' age for 2012–2016

4.2.1.3 Distribution of GeneXpert records by place

In epidemiology it is also common to describe participants according to place (BU SOPH [s.a.]). In this study visualizing the distribution of GeneXpert records by place also helps to show the extent to which the sample is representative of the population of GeneXpert positive patients in Swaziland.

Figure 4.3 shows that Manzini region contribute the most GeneXpert records across all the years 2012–2016, followed by Shiselweni, Hhohho and Lubombo respectively.

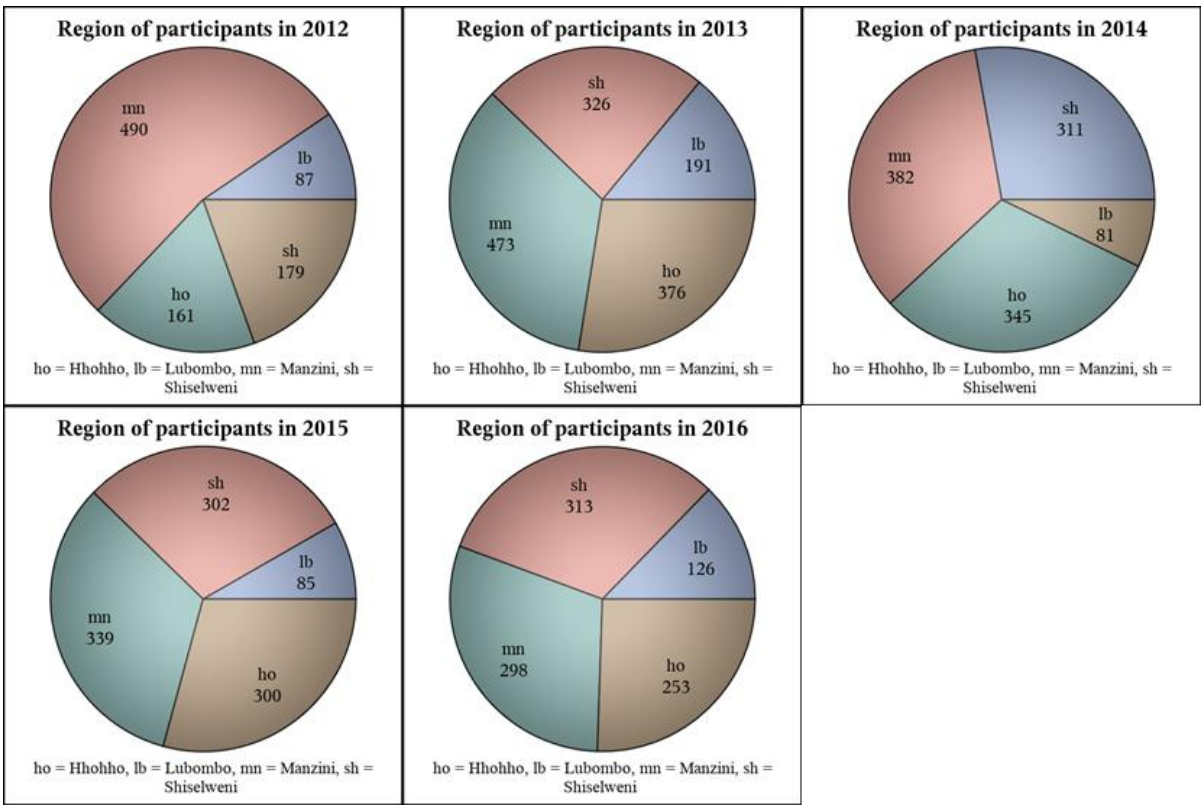


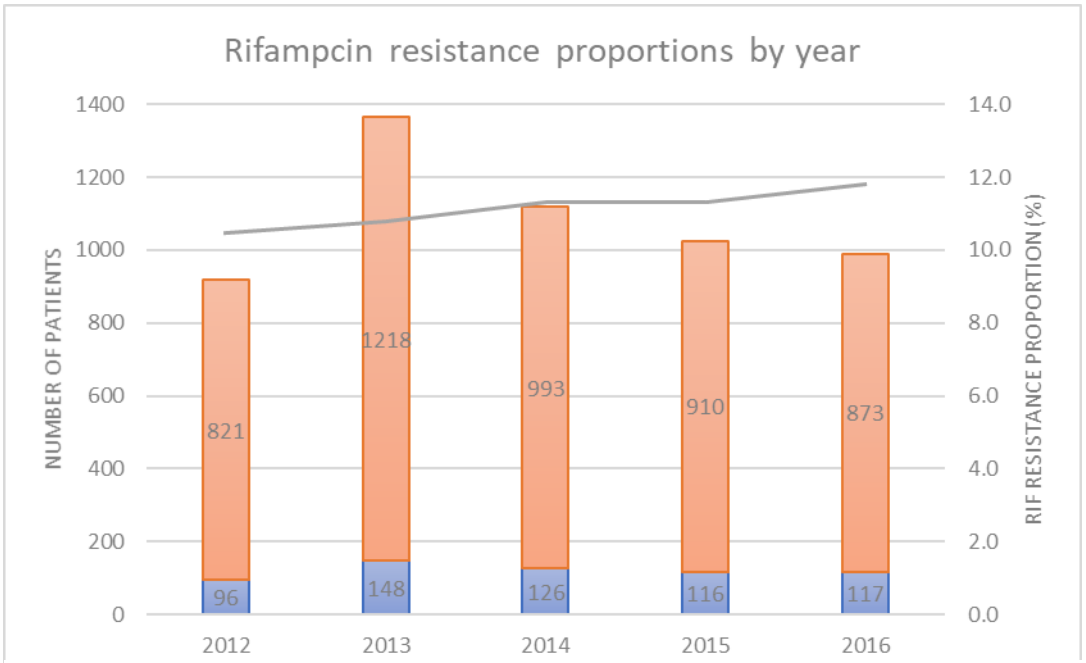
Figure 4.3 Contribution of each region to the sample for years 2012–2016

It is understandable for Manzini to have contributed the most since three sites were selected in Manzini as opposed to two sites in each of Shiselweni, Hhohho and Lubombo.

4.3 RIFAMPICIN RESISTANCE AMONG GENEXPERT RECORDS

The first objective of this study was to describe the prevalence of rifampicin resistance according GeneXpert with regard to person, time and place. The sections that follow present proportions of rifampicin resistance across the study period disaggregated by age group, gender and place.

4.3.1 Proportions of rifampicin resistance from 2012–2016



	2012	2013	2014	2015	2016
Resistant	96	148	126	116	117
Sensitive	821	1218	993	910	873
Proportion	10.5	10.8	11.3	11.3	11.8

Figure 4.4 Proportions of rifampicin resistance by year

Figure 4.4 above show the proportions of rifampicin resistance among persons from who GeneXpert records were obtained during 2012–2016 were 10.5%, 10.8%, 11.3%, 11.3% and 11.8% respectively. The number of observations in the sample was decreasing from 2013–2016. However, the proportion of rifampicin resistance remained rather stable between 10 and 12%. Exact 95% confidence intervals were calculated on each of the rifampicin resistance proportions as shown on Figure 4.4. The lower 95% confidence limits range from 8.6–9.9 while the upper 95% confidence limits range from 12.6–14.0.

95% confidence intervals have been included here because the aim of the study is to estimate the proportion of rifampicin resistance in the population of patients diagnosed of TB by GeneXpert. While the proportion of rifampicin resistance from each of the years is statistic that refers to the sample, 95% confidence interval estimates the proportion of rifampicin resistance in the population. Specifically, it is the interval within which the proportion of rifampicin resistance would fall 95% of the times if one repeatedly drew

samples from the GeneXpert records and determined the proportion of rifampicin resistance.

95% confidence intervals are also useful in this study for describing the trend of rifampicin resistance. The confidence intervals for each of the years 2012 – 2016 overlap which means that the proportions of rifampicin resistance are not significantly different from each other.

4.3.2 Description of rifampicin resistance by gender

4.3.2.1 Proportion of rifampicin resistance by gender in 2012

Of the 917 GeneXpert records analysed in in 2012, 909 had data on gender as shown in Table 4.5. About 0.9% (8/917) had no data on gender. Of the remaining 909, 52% were female while the reaming 48% were males. 12.1% of females had rifampicin resistant TB as compared to 8.6% of males. The meaning of the differing proportions of rifampicin resistance was explored through relative risk ratio. The relative risk of developing rifampicin resistant TB in females vs males was 1.39 (CI: 0.94–2.06). The confidence interval includes 1 which makes an outright conclusion that female gender is a risk factor for developing MDR TB implausible.

Table 4.5 Proportions of rifampicin resistance by gender in 2012

	Resistant	Sensitive	Total
Female	58	423	481
%	12.1	87.9	
Male	37	391	428
%	8.6	91.3	
Total	**95**	**814**	**909**

4.3.2.2 Proportion of rifampicin resistance by gender in 2013

1366 GeneXpert records were included for analysis in 2013 as shown in Table 4.6. 2.3% (32/1366) of them did not have data on gender. Of the remain 1334, 718 (54%), were female while the remaining 47% were male. The proportion of rifampicin is similarly slightly higher among females (11.1%) as compared to males (8.3%). The relative risk for

having rifampicin resistant TB among females vs males was 1.35 (CI: 0.96–1.88). Again, as in 2012 the confidence interval for relative risk encompasses one.

Table 4.6 Proportions of rifampicin resistance by gender in 2013

	Resistant	Sensitive	Total
Female	80	638	718
%	11.1	88.9	
Males	51	565	616
%	8.3	91.7	
Total	**131**	**1203**	**1334**

4.3.2.3 Proportion of rifampicin resistance by gender in 2014

1119 GeneXpert records were included in the analysis of 2014 as shown in Table 4.7. Eighteen (1.6%) had missing values for gender. Of the remaining 1101, 513 (47%) were females while the remaining 588 (53%) were males. The distribution pattern where there are slightly more males than females is a departure from the pattern seen in 2012 and 2013. However, the patter whereby the proportion of rifampicin resistance is slightly more among females than males remained the same. In 2014 12.3% of females had rifampicin resistant TB compared to 10% for males. The relative risk for having rifampicin resistant TB for females vs males was 1.22 (CI: 0.88–1.71) in 2014. An outright conclusion of whether relatively females have a higher risk of having rifampicin resistant cannot be made as the 95% CI includes 1.

Table 4.7 Proportions of rifampicin resistance by gender in 2014

	Resistant	Sensitive	Total
Female	63	450	513
%	12.3	87.7	
Male	59	529	588
%	10.0	90.0	
Total	**122**	**979**	**1101**

4.3.2.4 Proportion of rifampicin resistance by gender in 2015

In 2015, 1.6% (16/1026) of GeneXpert records had missing values for gender. Of the remaining 1010, 44% (447/1010), were female while the remaining 56% (563/1010) were

male as shown in Table 4.8. This pattern where the greater proportion of GeneXpert records were from males was a continuation of a pattern also seen in 2014. In terms of proportions of rifampicin resistance, the pattern whereby females have higher proportion is rifampicin resistance is maintained throughout 2015 as well. 13.4% of females in 2015 had rifampicin resistance compared to 9.1% of males. The relative risk ratio having rifampicin resistance for females vs males was 1.48 (95% CI: 1.04–2.11). Thus in 2015 it appears that having female gender predisposed persons from whom the GeneXpert records were obtained to having rifampicin resistance. However, it should be noted that 1.04 is not that far above 1

Table 4.8 Proportions of rifampicin resistance in 2015

	Resistant	**Sensitive**	**Total**
Female	60	387	447
%	13.4	86.6	
Male	51	512	563
%	9.1	90.9	
Total	**111**	**899**	**1010**

4.3.2.5 Proportion of rifampicin resistance by gender in 2016

The total number of GeneXpert records included for analysis in 2016 was 990. 1.8% (18/990) had missing values for gender. Of the remaining 972, 43% (417/972) were female and the remaining 57% (555/972) were male as shown in Table 4.9. This also marks a continuation of the pattern that was established from 2014. In terms of the proportion of rifampicin resistance as seen in all the years 2012–2016 females had a slightly higher proportion of rifampicin resistance compared to males. In 2016 12.2% of females had rifampicin resistant TB compared to 10.6% for males. The relative risk for having rifampicin resistance for females vs males was lowest in 2016 compared to the years 2012–2015. It was 1.15 with a 95% confidence interval of 0.81–1.64.

Table 4.9 Proportion of rifampicin resistance by gender 2016

	Resistant	Sensitive	Total
F	51	366	417
%	12.2	87.8	
M	59	496	555
%	10.6	89.4	
Total	**110**	**862**	**972**

4.3.3 Rifampicin resistance by age group

Next the proportions of rifampicin resistance were also disaggregated by age group because this is a useful way to understand the dynamics of anti-TB drug. In total between 2012–2016 a total of 5418 GeneXpert records were included for analysis of whom 338 (6%) had missing values for age.

In all the years 2012–2016 the highest number of GeneXpert records with rifampicin resistant TB was in the age group 25–44 as shown in Table 4.9. In 2012 the percentage of rifampicin resistant cases in the age group 25–44 was 72% (65/91). It was 63% (77/121), 67% (79/118), 67% (67/100), and 70% (73/104) for the years 2013–2016 respectively.

Table 4.10 Proportions of rifampicin resistance disaggregated by age group

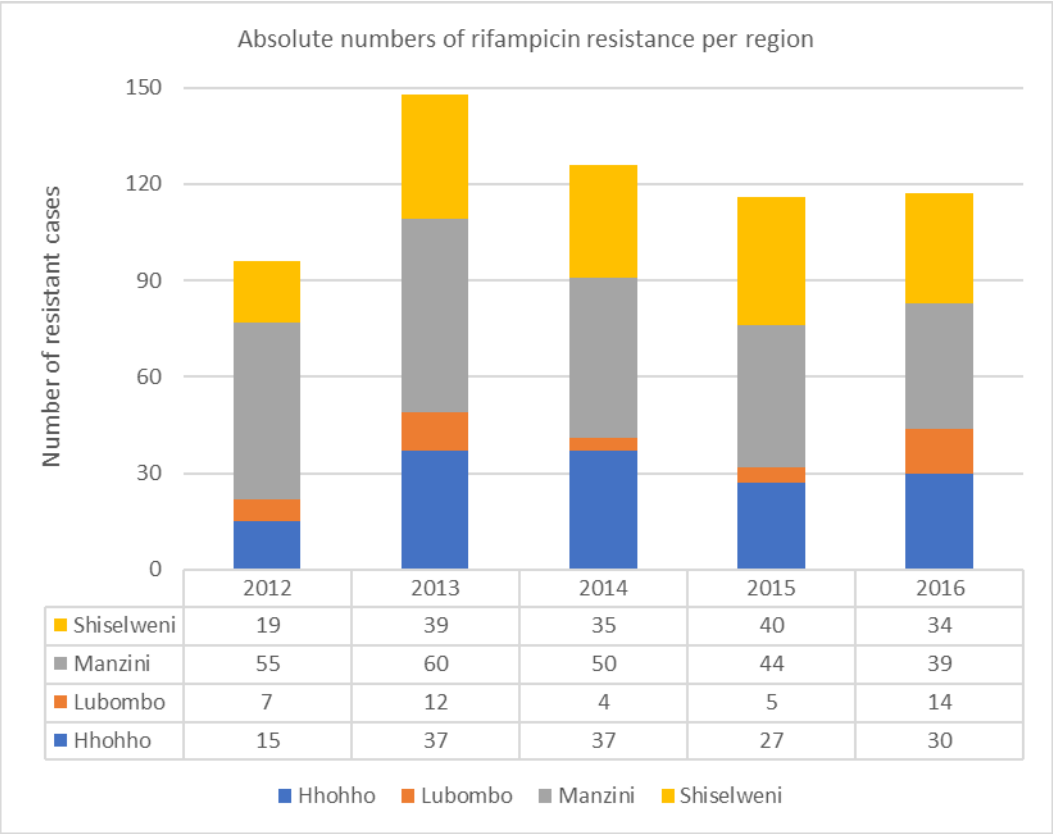
	2012		2013		2014		2015		2016	
	R	S	R	S	R	S	R	S	R	S
0–14	2	16	1	35	0	27	0	19	2	22
15–24	11	115	19	178	24	150	22	125	13	114
25–44	65	514	77	691	79	555	67	523	73	500
45–54	8	86	10	131	8	121	8	100	9	102
55–-64	2	32	9	80	3	52	3	34	6	55
65+	3	20	5	28	4	48	0	37	1	36
Total	**91**	**783**	**121**	**1143**	**118**	**953**	**100**	**838**	**104**	**829**

Only a maximum of 5.5% of the rifampicin resistant cases in each of the years 2012–2016 was coming from the combination of children under 15 and the elderly aged 65 and above. It was only in 2012 when 5.5% (5/91) of rifampicin resistant cases were from children and adults. In the years 2013–2016 the percentage of rifampicin resistant cases

from children and adults were 5.0% (6/12), 3.4% (4/118), 0% (0/100) and 2.9% (3/104) respectively.

4.3.4 Rifampicin resistance by region

Describing the epidemiological prevalence of rifampicin resistance also entails analysing proportions of rifampicin resistance by regions where patients reside. Figure 4.5 is a stacked bar chart showing the distribution of rifampicin resistant cases in each region for the years 2012–2016.



	2012	2013	2014	2015	2016
Shiselweni	19	39	35	40	34
Manzini	55	60	50	44	39
Lubombo	7	12	4	5	14
Hhohho	15	37	37	27	30

Figure 4.5 Distribution of 2012–2016 rifampicin resistant cases by region

4.3.4.1 Rifampicin resistance by place in 2012

In 2012, 96 out of the 917 (11%), GeneXpert records positive for MTB according to GeneXpert. The 95% confidence interval for that proportion ranges between 9–13. Most of the GeneXpert records with rifampicin resistance were from Manzini region at 57% (55/96). The smallest percentage was Lubombo region at 7% (7/96) while Shiselweni and Hhohho had 20% (19/96) and 16% (15/96) respectively. It should be noted that at each of Lubombo and Shiselweni regions one site had no data contributing to 2012 regional

data. This may partly explain the small number of GeneXpert records with rifampicin resistance was in Lubombo.

4.3.4.2 Rifampicin resistance by place in 2013

Out of the 1366 GeneXpert records which were positive for MTB according to the GeneXpert in 2013, 11% (148/1366), had rifampicin resistant TB with 95% CI between 9–13. Given that the confidence interval overlaps with that of 2012 one would conclude that there is no significant difference between the proportion of rifampicin resistance between 2012 and 2013.

Of the 148 GeneXpert records with rifampicin resistant TB, 26.4% (39/148) were from Shiselweni region while 40.5% (60/148), 8.1% (12/148), and 25.0% (37/148) were from the Manzini, Lubombo and Hhohho regions respectively. As in 2012 Manzini region has the highest proportion of rifampicin resistant cases, Lubombo has the lowest and that of Shiselweni and Hhohho are comparable.

By using the denominator of 148 which is the total number of rifampicin resistant cases in 2013 from all four regions, it has been demonstrated that there are differences in the contribution of each of the regions to the total burden of rifampicin resistance in Swaziland. However, it is also important to answer the question of whether the proportion of rifampicin resistance differs between regions. The proportions of rifampicin resistance within each region for 2013 are 9.8% (95% CI 7.0–13.3) for Hhohho, 6.3% (95% CI 3.3–10.7) for Lubombo, 12.7% (95 CI 9.8–16.0) for Manzini and 10.8% (95% CI 9.2–12.6). The 95% confidence intervals are overlapping across the years so that while the point estimates may look different, there is no statistically significant difference in the proportion of rifampicin resistance across regions.

This observation is also supported by chi-square statistic of 1.08 with 3 degrees of freedom and a p-value of 0.78. The chi-square statistic was calculated from a 4 by 2 contingency table where region was the row variable and rifampicin resistance was the column variable. As p-value is greater than 0.05 one concludes that the row and column variables are independent.

4.3.4.3 Rifampicin resistance by place for 2014

A total of 1119 GeneXpert records that were positive for MTB in 2014 and 126 (11.3%) of them had rifampicin resistant TB. This comparable to the proportions of rifampicin resistance for 2012 and 2013 which were 10.5% and 10.8% respectively.

Of the 126 rifampicin resistant cases 29.4% (37/126) were from Hhohho region; 3.2% (4/126), were from Lubombo region; 39.7% (50/126) were from Manzini region while the remaining 27.8% (35/126) were from Shiselweni region. As seen in 2012 and 2013 most cases were Manzini, the least number of cases was from Lubombo and the two regions of Shiselweni and Hhohho had almost the same number of cases.

At the level of the region the proportion of rifampicin resistance in 2014 was 10.7% (7.7–14.5) for Hhohho, 4.9% (1.4–12.2) for Lubombo, 13.1% (9.9–16.9) for Manzini and 11.3% (8.0–15.3) for Shiselweni. The proportion for Lubombo region is much lower than the proportions for Hhohho, Manzini and Shiselweni. Statistically the difference seen is not significant because of the confidence intervals for the proportions which overlap.

In addition, chi square statistic for a 4 by 2 table where the four regions were row variables and rifampicin resistance was the column variable, was 4.62 with 3 degrees of freedom and a p-value of 0.20. Since 0.20 is greater than the alpha of 0.05 it can be concluded that row variable and column variables are not related.

4.3.4.4 Rifampicin resistance by place in 2015

A total of 1026 GeneXpert records with results positive for MTB were included in the analysis. 116 (11.3%) had also rifampicin resistance. In terms of region of origin 23.3% (27/116), were form Hhohho region, 4.3% (5/116) were from Lubombo region, 37.9% (44/116), were from Manzini region and the remaining 34.5% (40/116) were from Shiselweni region. While it is still true that Manzini contributed the most cases and Lubombo the least, Shiselweni contributed a bit higher than Hhohho compared to previous years of 2012–2014.

At the level of each region the proportion of rifampicin resistance was 9.0% (95% CI 6.0–12.8) for Hhohho, 5.9% (95% CI 1.9–10.9) for Lubombo, 13.0% (95% CI 9.6–17.0) for

Manzini and 13.3% (95% CI 9.6–17.6) for Shiselweni. As seen in previous years the point estimate for the proportion of rifampicin resistance in Lubombo is much lower compared to those of Manzini, Shiselweni and Hhohho regions. However, the 95% confidence overlaps with the intervals for the other regions. Statistically it means that a conclusion that the proportion of rifampicin resistance in Lubombo is lower is not possible.

Chi-square statistic was also calculated for a 4 by 2 contingency table in which the regions constituted the row variables and rifampicin resistance represented the column variable. The chi square was 6.2 with 3 degrees of freedom and a p-value of 0.10. Since the p-value is greater than 0.05 it can be concluded that region and rifampicin resistance are independent of each other.

4.3.4.5 Rifampicin resistance by place in 2016

A total of 990 GeneXpert records with positive MTB results were included in the analysis for 2016. 117 (11.8%), of them had rifampicin resistance. According to the region of origin the 117 records were distributed as follows: 25.6% (30/117) were form Hhohho region, 12% (14/117) were from Lubombo region, 33.3% (39/117) were from Manzini and the remaining 29.1% (34/117) were from Shiselweni region. Thus in 2016 the number of GeneXpert records from Lubombo increased while those from each of Manzini, Hhohho and Shiselweni became comparable. Interestingly there were no cases of rifampicin resistance contributed by Lubombo F2 health centre in 2016.

At the regional level the proportions of rifampicin resistance were 11.9% (95% CI 8.2–15.8) in Hhohho, 11.1% (95% CI 6.2–18.0) for Lubombo, 13.1% (95% CI 9.5–17.5) for Manzini and 10.9% (CI 9.8–14.0) for Shiselweni. In 2016 the point estimates for the proportion of rifampicin resistance are comparable for all the regions including Lubombo. This is also corroborated by the overlapping of 95% confidence intervals for the regional proportions.

A chi square statistic was also calculated on a 4 by 2 contingency table with region as the row variable and rifampicin resistance as the column variable. The chi square was 0.8 with a p-value of 0.9. Since 0.9 is greater than the alpha of 0.05 it can be concluded that there is no relationship between region and rifampicin resistance.

4.3.5 Association between age group, gender, location with rifampicin resistance

In addition to chi square statistics, a logistic regression model was constructed provide a layer of determining if there exists any association between rifampicin resistance and factors that include region, age group and gender. According to Chi-square statistics already given above none of age group, gender and region seemed to be clearly associated with rifampicin resistance. To establish the validity of the observations above, a logistic regression model was constructed to provide a second layer of determining if there exists any association between rifampicin resistance and factors that include region, age group and gender.

Logistic regression provides an alternative approach to identifying risk factors for a dichotomous response outcome (Li 2013). It applies to this study because the dependant variable (rifampicin resistance), is a binary. Logistic regression predicts the odds of a GeneXpert record to turn out to have a rifampicin resistant strain given the age group to which they belong, or their gender or their region of origin when the other two factors are held constant. For the model rifampicin resistance = age-group gender sex, results of the model's global null hypothesis testing, and odds ratio estimates are presented below.

4.3.5.1 Global null hypothesis testing results

In SAS's proc logistic the null hypothesis whose results are presented in the section for global null hypothesis testing is one that assumes that none of the betas predicts the outcome of rifampicin resistance being equal to resistant (UCLA IDRE [s.a.]). The Chi-square statistic calculated by the likelihood ratio method is 43,9 with 10 degrees of freedom and a p-value of <0.0001. The p-values for chi square statistics calculated by Score and Wald methods also had p-values of <0.0001. This means that at least one of the betas is significant. Thus, values for analysis of odds ratios and Wald 95% confidence intervals are presented next.

4.3.6 Probability of developing rifampicin resistance by age group

4.3.6.1 Probability of developing rifampicin resistance for age group 0–14

GeneXpert records from persons aged 0–14 were 0.27 times less likely to have rifampicin resistance when compared with those aged 15–24 (see Table 4.11 below). Further GeneXpert records from persons aged 0–14 were also 0.26 times less likely to develop rifampicin resistance compared to those from the age group 25–44.

On the other hand, there did not seem to be any differences between the probability of developing rifampicin resistance for GeneXpert records from persons in the age group 0–14 and those in age groups 55–64 and 65 +.

4.3.6.2 Probability of developing rifampicin resistance for age group 15–24

GeneXpert records from persons aged 15–24 were 1.49 times more likely than those from persons aged 45–54 to develop rifampicin resistance (see Table 4.11 below). On the other hand, there does not seem to be any differences in the probability to develop rifampicin resistance for age group 15–24 when compared with age groups 25–44, 55–64 and 65 +.

4.3.6.3 Probability of developing rifampicin resistance for age group 25–44

GeneXpert records from persons aged 25–44 were 1.56 times more likely to develop rifampicin resistance compared to those aged 45–54. Further as indicated by Wald 95% confidence intervals that include 1, there does not seem to a difference between the probability of developing rifampicin resistance between age group 25–44 and 55–64 or 65+.

4.3.6.4 Probability of developing rifampicin resistance for age group 45–54

The probability of developing rifampicin resistance did not seem to be different between GeneXpert records from persons in the age groups 45–54 and 55–64 or 65+.

4.3.6.5 Probability of developing rifampicin resistance for age group 55–64

The probability of developing rifampicin resistance did not seem to be different between GeneXpert records from persons in the age group 55–64 and 65+.

4.3.7 Probability of developing rifampicin resistance by gender.

Females were 1.31 times more likely to develop rifampicin resistance than males.

4.3.8 Probability of developing rifampicin resistance by region

GeneXpert records of persons from Hhohho were 0.75 times less likely to have rifampicin resistance than those from Manzini. However, there was no difference in the probability of developing rifampicin resistance for persons of persons from whom GeneXpert records were obtained living in Hhohho compared to Lubombo or Shiselweni.

Persons from GeneXpert records were obtained living in Lubombo were 0.62 and 0.65 times less likely to have rifampicin resistance than those from Manzini and Shiselweni respectively. Lastly, there was no difference in the probability of having rifampicin resistance between GeneXpert records from person living Manzini and Shiselweni.

Table 4.11 Odds ratio estimates and Wald confidence intervals for likelihood of various age groups, gender and region to influence rifampicin resistance

	Estimate	95% Confidence Limits	
0–14 vs 15–24	0.267	0.096	0.741
0–14 vs 25–44	0.255	0.093	0.696
0–14 vs 45–54	0.398	0.140	1.134
0–14 vs 55–64	0.342	0.115	1.013
0–14 vs 65 +	0.397	0.126	1.253
15–24 vs 25–44	0.955	0.744	1.227
15–24 vs 45–54	1.492	1.014	2.195
15–24 vs 55–64	1.281	0.788	2.082
15–24 vs 65 +	1.489	0.807	2.746
25–44 vs 45–54	1.562	1.122	2.174
25–44 vs 55–64	1.341	0.860	2.090
25–44 vs 65 +	1.559	0.874	2.779
45–54 vs 55–64	0.858	0.505	1.458
45–54 vs 65 +	0.998	0.523	1.905
55–64 vs 65 +	1.163	0.572	2.364
Female vs Male	1.310	1.089	1.577
Hhohho vs Lubombo	1.208	0.828	1.762
Hhohho vs Manzini	0.752	0.595	0.951
Hhohho vs Shiselweni	0.789	0.610	1.019
Lubombo vs Manzini	0.623	0.436	0.890
Lubombo vs Shiselweni	0.653	0.451	0.946
Manzini vs Shiselweni	1.048	0.838	1.312

4.4 ANALYSIS OF MECHANISMS OF RESISTANCE

4.4.1 Missing deep machine data

This part of the of the results seeks to describe the patterns of mutations identified by GeneXpert in the years 2012–2016. The data answering these questions is contained in deep machine data from the GeneXpert instrument. It was only possible to obtain this data for three sites namely Manzini F1, Manzini F2 and Shiselweni F2 and this data will be presented below.

Deep machine data could not be obtained for different reasons. For instance, in Hhohho F2, Shiselweni F1, Hhohho F1, Manzini F3 and Lubombo F2 the data was not available because the instrument had been upgraded from GeneXpert IV to GeneXpert XVI model.

The new instruments thus did not contain enough data from the study period. In Lubombo F1 on the other hand GeneXpert machine had not been changed but the data from previous years had been deleted from the machine as it had become slow because of data overload.

4.4.2 Approach to analysis of available deep machine data

The available probes are analysed per site. The period of analysis is 2012–2016 combined. This is to avoid making proportions of probes where the denominator is too small.

4.4.3 Mutation patterns at Shiselweni F2 for 2012–2016

Table 4.12 Mechanism and loci of mutation at Shiselweni F2 2012–016

	Locus of mutation					
	A	**B**	**C**	**D**	**E**	**Total**
Delay	0	4	0	5	7	16
Column %	0.0	15.4	0.0	6.9	3.5	
Dropout	5	22	9	67	196	299
Column %	100.0	84.6	100.0	93.1	96.6	
Total	**5**	**26**	**9**	**72**	**203**	**315**

A total of 315 rifampicin resistant cases were found at Shiselweni F2 between 2012–2015. This data is displayed in Table 4.12 above. The most common mechanism in which resistance manifested was dropouts whereby a probe would not hybridize at all. Dropout was the single mechanism behind resistance in Probes A and C. For Probes B, D and E, in a minority of cases the mechanism behind resistance was delay.

Mutations in Probe B accounted for resistance in 26 cases of which 4 were caused by delays while the remaining 22 were caused by dropouts. Mutations in probe D accounted for 72 cases of which 5 were caused by delays and 67 were caused by dropouts. Lastly, mutations in probe E accounted for 203 cases comprising 196 dropouts and 7 delays. Of the 315 resistant strains, the majority had mutation in Probe E with 64.4% (203/315) and probe D with 22.9% (72/315). Probes A, B and C had 1,6% (5/315), 8.3% (26/315) and 2.9% (9/315) respectively.

4.4.4 Mutation patterns at Manzini F2 for 2012–2016

Table 4.13 Mechanism and loci of mutation at Manzini F2 2012–2016

	Locus of mutation					
	A	**B**	**C**	**D**	**E**	**Total**
Delay	0	3	1	8	2	14
Column %	0.0	17.7	50.0	16.3	2.3	
Dropout	2	14	1	41	87	145
Column %	100.0	82.3	50.0	83.7	97.7	
Total	**2**	**17**	**2**	**49**	**89**	**159**

A total of 119 rifampicin resistant strains were included from Manzini F2. The distribution of the mechanisms behind and the loci of mutations is shown in Table 4.13. The most frequent mechanism causing resistance was dropout as seen in the case of Shiselweni F2.

Dropout was the single mechanism behind resistance in probe A where there were 2 (1.3%), strains with mutations in probe A. In Probes B–E both delays and dropouts were involved in causing resistance, and similarly to the pattern from Shiselweni F2 dropouts accounted for resistance in a greater proportion of strains than delays.

As seen from the column percentage in Table 4.13 delays accounted for 17.7% (3/17) of strains in which mutation(s) were located in probe B, 50% (1/2) of strains in which mutations were located in Probe C, 16.3% (8/49) of strains in which the mutations were located in probe D and 2.3% (2/89) of strains in which the mutation(s) were located in probe E. On the other hand, dropouts accounted for 82.3% (14/17), 50% (1/2), 83.7% (41/49), and 97.7% (87/89) of strains in which the mutation(s) were in probes B, C, D and E respectively.

4.4.5 Mutation patterns at Manzini F1 for 2012–2016

Table 4.14 Mechanisms and loci of mutation at Manzini F1 2012–2016

	Locus of mutation					
	A	**B**	**C**	**D**	**E**	**Total**
Delay	0	1	0	3	3	7
Column %	0.0	12.5	0.0	10.3	3.9	
Dropout	1	7	4	26	74	112
Column %	100.0	87.5	100.0	89.7	96.1	
Total	**1**	**8**	**4**	**29**	**77**	**119**

A total of 119 strains with rifampicin resistance were included from Manzini F1 for the period 2012–2016. Table 4.14 represents the distribution of mechanisms and loci of mutations in the 119 strains. Similarly, to the patterns seen in Nhlangnano and Manzini F2, the mechanism behind most of the resistance is dropouts.

In Manzini F1 dropout was the single mechanism behind resistance in the (1/1) strain in which the mutation(s) were in Probe A and (4/4) strains in which the mutation was in probe C. In probes B, D and E both delays and dropouts were mechanism of resistance. Dropouts accounted for a greater proportion of resistant strains that delays.

As illustrated by column percentages delay was the mechanism in 12.5% (1/8), 10.3% (3/29), and 3.9% (3/77) of strains in which the mutation(s) were in probes B, D and E respectively. On the other hand, dropout was the mechanism in 87.5% (7/8), 89.7% (26/29), and 96.1% (74/77) of strains in which the mutations were in probes B, D and E respectively.

4.5 SUMMARY

This study analysed the 5418 GeneXpert records drawn from 9 facilities in the 4 regions of Swaziland. The sample consisted of slightly more males than females resulting in a male: female ratio of 1.06 for TB infection. This study found that the proportion of rifampicin resistance varied between 10,5%–11,8% for the years 2012–2016. But as opposed to Tb infection, the male: female ratio for rifampicin resistant TB infection was reversed, at 0.82 : 1. However, proportion of rifampicin resistant TB were found to be

similar between the regions despite the fact that Manzini contributed more patients with rifampicin resistant TB while Lubombo contributed the least.

The study also found that although age group, gender and place of living did not seem like risk factors for rifampicin resistance, there possibly were nuances. For instance, the study found that GeneXpert records from persons aged 15–24 and 25–44 were respectively, 1.36 and 1.56 times more likely to have rifampicin resistant TB than those aged 45–54.

Lastly, the study determined that dropouts were the more common mechanism behind resistance compared to delays. Further, mutations in Probe E were the most common cause for rifampicin resistance in the three sites for which deep machine data was available. In the next chapter, the findings are discussed and interpreted, leading to some recommendations for policy, practice and further study.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 INTRODUCTION

The previous chapter presented the findings of this study. this chapter features a discussion of the results. The purpose of this chapter is to describe what the results presented in Chapter 4 may mean in the light of the Swaziland context and what has been found in other studies. The purpose of this was to retrospectively determine the epidemiological prevalence of rifampicin resistance in Swaziland during the years 2012–2016. In addition, study also aimed to determine the proportions of probes in which GeneXpert detected mutations. The study was successful in determining the epidemiological prevalence of rifampicin resistance. However, for the proportion of probes only three centres had the data available making it difficult for the study to fulfil the second purpose adequately.

The chapter begins with a brief review of the research design which is followed by a summary of the findings. Then for simplicity, the structure of the chapter is such that results are discussed in the order in which they are presented in the Chapter 4.

5.2 RESEARCH DESIGN AND METHOD

GeneXpert has been widely implemented in many countries. As reported by the WHO, 130 of the 145 high burden developing countries eligible for concessional prices for GeneXpert instruments had implemented GeneXpert by 2016 (WHO 2016b). At the same time there remains a gap in the reporting of anti-tuberculosis drug resistance to the global anti-tuberculosis surveillance project Zignol et al 2016:[1083]). This study employed a descriptive correlational design to describe the epidemiological prevalence of rifampicin resistance in Swaziland for 2012–2016. Also, the study sought to describe the molecular patterns of resistance according to GeneXpert probes.

Patients who tested positive for GeneXpert between the years 2012–2016 were eligible to be included in the study. Multistage sampling was employed whereby in the first stage

9 sites were purposively selected from the four regions of Swaziland. Then, in the second stage fifty percent of eligible patients at each site were randomly selected into the study sample. For each patient in the sample age, sex, region of origin, and rifampicin resistance results were collected for analysis of the epidemiological prevalence of rifampicin resistance. Then, for Shiselweni F2, Manzini F1 and Manzini F2 deep GeneXpert machine data was also extracted for the analysis of GeneXpert probes.

Frequencies of rifampicin resistance with confidence intervals were calculated using SAS studio version 9.04M6 for the years 2012–2016, age groups and regions. Chi-square and proc logistic were used to test relationships between rifampicin and independent variables namely age group, gender, and place. About rifampicin resistant observations, the proportion of resistance due to either delays or dropouts was calculated for Shiselweni F2, Manzini F1 and Manzini F2. Also, the frequencies of probes in which the mutations were located were determined.

5.3 SUMMARY AND INTEPRETATION OF RESULTS

5.3.1 Summary and interpretation of study findings

This study found that males outnumbered females in terms of TB infection at a male: female ratio of 1.07 : 1. But in terms of rifampicin resistant TB infection it was females who outnumbered males, such that the male: female ratio for rifampicin resistant TB was 0.86:1. About the prevalence of rifampicin resistance, this study found that for 2012–2016 the proportions of rifampicin resistance were stable in a narrow range of between 10.5–11.8%. Further, rifampicin resistance was more prevalent among GeneXpert records from persons aged 25–44 and less prevalent among children aged, 0–15 and the very old, aged 65 and above. In terms place Manzini region had the highest number of GeneXpert records with rifampicin resistant TB.

Chi-square computed on yearly samples suggested that there was no relationship between rifampicin resistance any of age group, gender or region. However logistic regression for the combined sample showed that there may be some significant associations. Specifically, logistic regression showed that GeneXpert records from persons aged 15–24 and those aged 25–44 were respectively 1.49 and 1.56 times more

likely to have rifampicin resistance than those aged 45–54. In addition, females were more likely than males to develop rifampicin resistance.

About mechanisms of rifampicin resistance, this study found that dropouts were the most common mechanism behind resistance. That is to say that it was more common to find a missing probe in the event that rifampicin was present, than it was to find that all probes present with delay in the hybridization of the probe in which the mutation lay. Further, this study determined that probe E was the most common locus of mutations detected by GeneXpert in Swaziland during 2012–2016.

The descriptive correlational was suitable for this study because it allowed the collection and analysis of a large amount of data to describe prevalence and to test associations between variables. However, as the study depended on previously data it was not possible to obtain data on other risk factors for TB and drug resistant. For example, in this study it was impossible to get data on the treatment history of patients and other behaviours such as smoking, alcohol consumption and health seeking behaviours.

Still, this study can be considered to have fulfilled its main objective which was to describe the epidemiological prevalence of rifampicin TB according to GeneXpert. By so doing, this study also demonstrate that it is possible to use the GeneXpert to provide national data on rifampicin resistance. The study fare less well in terms of describing the pattern of resistance according the probes of GeneXpert. This is mainly because it was impossible to obtain deep machine data for most of the centres involved.

5.3.2 Social construction of technology and this study's findings

Bijker (2001:15523), traces as unfortunate the roots of social construction of technology in the challenge to technological determinism. However, for this study this beginning for SCOT was a logical departing point. This is because for the development of GeneXpert from a being a real time technology for the detection of anthrax in the United States postal services experienced pressure from relevant social groups to evolve to a rapid diagnostic for the over thirty-five diseases which the GeneXpert platform now diagnoses. Specifically, this study would belong to a relevant social group interested in interpreting GeneXpert as a technology suited for epidemiology of rifampicin resistance namely epidemiological prevalence and description of patterns of rifampicin resistance.

Therefore, this study contributes to SCOT in the sense that while GeneXpert is considered by its developers and other relevant social groups as a rapid diagnostic for TB and rifampicin resistance, this study argues that it is also a tool that can be used for epidemiological purposes.

5.4 MISSING DATA

Missing data has been selected as a starting point for discussion because missing data may negatively impact representativeness of data and accuracy of the proportions of rifampicin resistance seen. Thus, ideally the proportion of missing data must be small so that the impact on accuracy is minimal. In addition, the missingness of the data should also be considered. In the case of this study, missing values on rifampicin resistance, age and gender were assumed to be missing at random. That is, the missing of rifampicin resistance from an observation was not related to that observation's value of rifampicin resistance.

In fact, the missing values were probably related to clerical omissions. This may be so because in facilities like Lubombo F1, Hhohho F2 a microscopy register was used to capture the results of GeneXpert. However there did not seem to be a standard way in which this adaptation was being implemented. In Lubombo F1 at first, the abbreviations H, M, L, VL and N were used to denote GeneXpert MTB results of "MTB Detected, High", "MTB Detected, Medium" "MTB Detected, Low", "MTB Detected, Very Low" and "MTB Not Detected" respectively. Rifampicin resistance would only be included if the it was detected or indeterminate.

On the other hand, in Hhohho F2 results of GeneXpert were written in full including the rifampicin resistance results. Thus, when the result of rifampicin was missing the one would consider that it is missing.

In Shiselweni F2 Shiselweni F1, Manzini F1 and Manzini F2 a register was designed specifically to capture GeneXpert results in both paper and electronic forms. In those registers there was a field to capture the GeneXpert MTB result and another field for capturing the rifampicin result. But clerical errors still occurred whereby the result of MTB is indicated but not that of rifampicin resistance.

In Hhohho F1, Manzini F3 and Lubombo F2 results of the GeneXpert were captured using a Laboratory information system. Missing data for rifampicin resistance was associated with the beginning stages of the LIS implementation whereby operators would enter the results of GeneXpert manually into the LIS. This problem became less common once the LIS was set up to automatically pick up GeneXpert results automatically.

The method chosen to deal with missing rifampicin results was pairwise deletion. This is whereby an analysis makes use of all non-missing pairs for analysis. The result is that different computations have different denominators. For example, in this study after deleting the 248 GeneXpert records with missing rifampicin results there were 348 missing values ages and 92 missing values for gender. Pairwise deletion was appropriate in this case because the proportion of missing data is small (less than 5%), and the missingness of the data was assumed to be missing at random. Other techniques that can be employed to missing data include listwise deletion and multiple imputation.

5.4.1 Possible methods to eliminate missing rifampicin resistance data in future

If GeneXpert data is to be used for surveillance of rifampicin resistance, missing data must be kept to a minimum. More so, rifampicin resistance data should not be missing. Thus, below the discussion turns to recommendation on how to minimize missing rifampicin resistance data.

5.4.1.1 Manual systems

In manual recoding systems a GeneXpert results register should be developed with dedicated fields for capturing the main elements of a GeneXpert result. The key is for the fields in the GeneXpert register to be exhaustive, covering all the possible results from GeneXpert. This includes fields to record inconclusive results such as errors and valid results. For errors additional fields may be required for recording the error code and the module on which the error was obtained.

While the above measures may ensure uniform and easy recording of data across sites, it is periodic supervisory data checks that will ensure that omissions in data are early identified and remedied. Once this is satisfied, old registers must be stored according to data retention policies of the Swaziland Health Laboratory Services.

5.4.1.2 Electronic systems

The researcher was made to understand that rifampicin resistance data was missing in cases where the operators had to enter the results in the system manually. Thus, for LIS it is important to ensure the LIS automatically abstracts data from the instrument.

5.4.1.3 Deep machine data

Data retention policies of the SHLS should be extended to include deep machine data. In this study the researcher could not access deep machine data from 6 sites evidencing how the management of such data is not following current data retention policy whereby old registers should be preserved for ten years.

By extension the researcher would expect that if the data retention is extended to deep machine data, the policy would stipulate a period of ten years for the retention of deep machine data. Further, it would also be useful if the policy would also address issues of on-site and off-site backup.

It should also be mentioned that GeneXpert instruments in Swaziland are connected to Gx-Alert system. The function of Gx-Alert which is closely related to surveillance is the ability of Gx-Alert to abstract deep machine data from all GeneXpert machines in laboratory network. Regrettably Gx-Alert does not back up such data, so that once the data is deleted on the physical machine it also deleted on Gx-Alert.

A functionality where Gx-Alert can keep the historical deep machine data of each site would be desirable. It would simplify data collection for purposes of surveillance. However, with Gx-Alert in its current form a script could be implemented which provides a periodic report of rifampicin resistance proportions by person and place for given periods of time.

5.4.2 Missing data for treatment history

It was puzzling to realize that all observations in the dataset had missing values on treatment history. Having a history of previous TB treatment is a known risk factor for developing rifampicin resistant TB. For instance, Sanchez-Padilla, Dlamini, Ascorra, Rüsch-Gerdes, Tefera, Calain, De la Tour, Jochims, Richter and Bonnet (2012:[31,32]) report that in a survey conducted in Swaziland in 2010, the proportion of MDR was about four times greater among patients with history of previous TB treatment than among new patients in Swaziland (33.8% vs 7.7% respectively). A finding of a similar pattern when describing rifampicin resistance by GeneXpert would have further strengthened the case for using GeneXpert to describe the epidemiology of rifampicin resistance.

Two factors which may have contributed to the missing treatment history values are non-availability of data collection tools adapted to collecting treatment history data and the workflow of TB diagnosis in Swaziland. The two important data tools at the level of the laboratory are the laboratory examination request form and the laboratory register.

It is notable that as part of its role of providing guidance to national TB control programs, WHO suggests and regularly updates tools used by national programs. As far as laboratory examination request forms and registers are concerned, the concept of treatment history disaggregated data took shape can be traced to 2008 and was fully developed in 2013. This discussion illustrates this by comparing tools suggested in 2006, 2008 and 2013.

The models for the laboratory examination request form and the laboratory register suggested in WHO guidelines in 2006 lack completely any fields to classify patients as new or previously treated. Instead examination requests were classified according to two reasons for examination namely diagnosis and follow up monitoring of TB treatment. Under this regime new patients including those with history of TB treatment fell under the reason for examination equal to diagnosis (WHO 2006:[9, 11]).

A precursor of disaggregating diagnostic patients into new and previously treated can be traced to the 2008 emergency update of guidelines for the programmatic management of drug resistant TB (WHO 2008:227, 228). In that update, the request form maintained the dual classification of the reason for examination but a note in the lab register suggested

in that same update at least acknowledged that diagnosis patients included “New patients or patients starting a retreatment regimen”.

With enough training of clinical staff who were responsible for completing laboratory examination request forms, the models suggested in 2008 could have ensured the availability of the data missing in the study’s dataset. However why this update may not have resulted in some treatment history disaggregated data will be explained in the section on workflow.

A clear treatment history disaggregated suggestion for the laboratory examination request form and register was suggested by WHO in 2013. In both documents there was space to capture the treatment history of the patient so at the level of the lab comparison would be possible of the proportions of rifampicin resistance between new and previously treated diagnostic cases (WHO 2013b:25, 27, 28).

Of course, at the level of the clinic data was already somehow captured disaggregated by treatment even from the guidelines of 2006. Thus, for the national Tuberculosis control program there was always a way to analyse and compare the proportions of drug resistance between new patients and previously treated cases who start treatment. Thus, the advantage of the possibility for that same analysis to be done at the level of the laboratory is that the analysis can reflect better the Eswatini epidemiological situation. This is because data at clinic level reflects only those biologically confirmed TB patients who started treatment while that at the laboratory reflects all biologically confirmed TB patients.

The bias introduced by considering patients who start treatment becomes more visible when one considers the missingness of the patients who do not start treatment. It is most likely that those who do not start treatment after being diagnosed are not random patients rather there may be patterns. For instance, patients with more severe disease may die before starting treatment. Severe disease may be linked with delays in seeking health care as is common among males.

However, a comparison of the WHO updates of laboratory examination request forms and registers with the corresponding tools in Swaziland reveals that the national program did not keep up with the changes. A review of laboratory examination request forms from

Swaziland shows that before 2012 a dedicated form was being used for requesting TB laboratory examinations. The form is attached in annex 1A. The form follows the suggested model of presented in WHO (2008:227) guideline. Thus, it had no dedicated fields for capturing treatment history disaggregated data.

In 2012 a new general laboratory examination request form was introduced (Annexure 1B). It also lacked specific fields for disaggregating diagnostic TB patients into new and previously treated. Even an update on the same form in 2016 is still not compliant with collecting treatment history disaggregated data.

If GeneXpert data is to be used for surveillance of rifampicin resistance, then It is necessary that the laboratory request form and LIS must be updated to capture the TB treatment history of diagnostic cases.

5.5 PROPORTIONS OF RIFAMPICIN RESISTANCE

5.5.1 Age characteristics of GeneXpert records

This study finds that there is a differential risk for differing age groups for developing rifampicin resistant TB. Further, most patients with TB and rifampicin resistance was in the age group 25–44. This is to be understood in the partly context of the population structure of Swaziland of which Index Mundi (2018) reports that the age group 15–35 comprises about 35% of the Swazi population. This is also echoed by the GKoS (2015:[49]), when they narrate that about 4 in 5 people in Swaziland are below the age of 35, of whom one third are aged 15–35. At the same time, the elderly who are aged more than 65 years only constitute about 4% of the population.

Another possible reason for most TB cases to concentrate in the age group 25–44 may lie in the epidemiology of HIV in Swaziland. TB infection in general may also be thought of as being influenced by the HIV infection. In Swaziland the prevalence of HIV was reported to be 31% among patients aged 18–49 (Mchunu, Griensven, Hinderaken, Kizito, Sikhondze, Manzi, Dlamini and Harries 2016:105). As 25–44 is part of the age group most affected by HIV, it is plausible that TB infection among them is also influenced by HIV infection. More so when the fact that most (73%), TB patients notified in 2014 were also infected with HIV (GKoS 2014).

This study's finding that the youth are most affected is significant because TB among youths threatens the prospects of Swaziland benefitting from the country's demographic dividend. According to GKoS (2015:[2]), declining fertility rates mean that in the not distant future Swaziland will have more economically productive persons than dependants. But these possible benefits are threatened by the dual epidemic of HIV and TB among the youths who form most of the population.

Therefore, it is commendable that there is a national youth policy (GKoS 2009), which addresses HIV among the youth through integration of age appropriate sex education into education curriculums. But as HIV and TB constitute a dual epidemic and whereby both diseases affect the youth, efforts must be made for the national youth policy to advocate that both HIV and TB awareness be integrated into the country's education curriculum.

This study also finds lower numbers of TB and resistant cases in the 0–14 age group. This is consistent with trends observed in global data whereby data is shaped like a 'J' as Bierrenbach (2010:[2]) describes. Notably TB among children aged 0–4 is indicative of recent transmission. In this study about one fifth (26/124 data not shown) of TB cases in the 0–14 age group is from children in the 0–4 age group. What is worrying is that all but one is rifampicin resistant. This illustrates a need to strengthen infection control measures in the homes of patients.

5.5.2 Discussion on the proportions of rifampicin resistance for 2012–2016

This study's findings show a relatively high proportion of rifampicin resistance among diagnostic cases in Swaziland. The proportions ranged between 10–12% for across 2012–2016. This is unlike the findings of Ismail, Mvusi, Nanoo, Dreyer, Omar, Babatunde, Molebatsi, Van der Walt, Adelekan, Deyde, Ihwekweazu and Madhi (2018:784) in a recent national representative survey of drug resistant TB in South Africa. They found an overall rifampicin resistance prevalence of 4.6% for participants enrolled between 2012–2014.

However, that South Africa would have a lower proportion of rifampicin resistance than Swaziland is not surprising. In fact, even Sanchez-Padilla et al (2012:[31, 32]), in an anti-TB drug resistance survey found that proportions of MDR which were higher compared

to South Africa and Mozambique. The overall proportion of MDR in the Sanchez-Padilla et al's (2012) study was 19.3% for new and previously treated cases aged more than 14 years combined. Thus, the findings of this study are mostly likely a true reflection of the rifampicin resistance situation in Swaziland. The differences between 19.3% and the 10–12% seen in this study could be accounted for by sampling differences between the present study and that of Sanchez-Padilla et al (2012).

There are several important sampling differences between the present study and the Sanchez-Padilla study. First, the present study included GeneXpert records with positive GeneXpert results while that of Sanchez-Padilla et al (2015) include diagnostic patients with positive smears. This difference matters because some GeneXpert records with negative smears could still be GeneXpert positive. For instance, Chemhuru, Duka, Nanan-n'zeth, Simons, Van den Broucke, Fajardo and Bygrave ([s.a.]) find that Ziehl Nielsen smear microscopy detected only about 60% of the cases detected by GeneXpert. Thus, the present study included variability from the GeneXpert records which would have been excluded in the Sanchez-Padilla et al's (2012), study.

Second, the present study included GeneXpert records from persons aged 0–14 who were excluded in the Sanchez-Padilla study. Besides issues of consent, it is also known that TB is more difficult to detect in children. The difficulties in detecting TB in children may in part account for the difference seen in the proportion of rifampicin resistance in the present study and that seen in the study of Sanchez-Padilla et al (2012).

A third important factor that contributes to the higher proportion overall rifampicin resistance is that GeneXpert is known to be unable to identify resistance in strains in which resistance is conferred from outside the rifampicin resistance determining region. As discussed in Chapter 2, GeneXpert identifies mutations in the RRDR region. Over 96 percent of strains of rifampicin resistant strains have mutations in this region. Only a very small percentage of strains have their rifampicin resistance conferring mutations elsewhere.

The small percentage of resistant strains with conferring mutations outside the RRDR would not significantly affect the sensitivity of GeneXpert to detect rifampicin resistance if not for the fact some geographical regions carry a disproportionate burden of those rare strains. Sanchez-Padilla et al (2015:[1181]) found that 38 percent of rifampicin resistant

strains in Swaziland carried a rare mutation outside the RRDR. The mutation so called I572F lies just outside the region of codons 507–533 in which GeneXpert detects. This means that in Swaziland GeneXpert has reduced efficiency for detecting rifampicin as it stands to miss at least 30% of the strains.

Even though the findings of Sanchez-Padilla et al (2015) reflect significantly higher overall proportion of rifampicin resistance the results of the present study are still plausible. Therefore, the present study demonstrates that GeneXpert can produce reliable results when used for describing proportions of rifampicin resistance. This is important to establish given that part of what the present study suggests is that GeneXpert data can be used for continuous surveillance of rifampicin resistance.

5.5.3 Trends in the proportion of rifampicin resistance with time

In the present study, there was no discernible trend in the proportion of rifampicin resistance by year. The absence of a discernible trend is illustrated by overlap in the confidence intervals of the proportions of rifampicin resistance for each of the years 2012–2016. The confidence intervals are 8.6–12.6, 9.2–12.6, 9.5–13.3, 9.4–13.4 and 9.8–14.0 for the years 2012–2016 respectively. There is no clear reason why this is so, but it is notable that Ismail et al (2018:784) also found no difference in the proportions of MDR between their study and a baseline drug resistance survey conducted in 2001 (2.8% in 2012–2014 vs 2.9% in 2001–2002). This may suggest that changes in the epidemiological situation of rifampicin resistance a country take time.

5.5.4 Proportions of rifampicin resistance by gender.

The findings of this study show that the there is no statistically significant differences in likelihood of males versus females developing drug resistant TB. A closely related ratio that is widely discussed in literature is M:F ratio. For the present study, the M:F ratios are 1.07:1 and 0.82:1 for MTB infection and rifampicin resistance respectively. The M:F ratio whereby females are more prone to rifampicin resistant TB is also reflected in the finding of an odds ratio of 1.31 by logistic regression.

The M:F ratio for TB infection found in this study is lower compared to global M:F ratios of 2.1:1 reported by the WHO in 2017 (WHO 2018a:6). As Horton, MacPherson, Houben,

White and Corbett (2016:[10]) observed it is normal for countries with high HIV prevalence to have M:F ratios that are lower. For instance, M: F estimates from Zimbabwe and South Africa which has a high HIV prevalence was 0.67.

The researcher expected that that the M:F ratios would at least be the same for MTB infection and rifampicin resistant TB, but results show that M: F for rifampicin resistant TB is lower. There is a paucity of articles on the link between gender and drug resistant TB but for TB infection, risk factors fall into two broad classes namely behavioural and physiological. Behavioural factors include smoking, alcohol consumption, imprisonment and undernutrition which one can rightly expect to be associated more with males than females. More so in Africa where societies are more patriarchal. On the other hand, part of the physiological factors has to do with how the immune system is regulated by sex hormones.

The researcher had expected that some behavioural practices should foster the development of TB such as non-adherence and treatment interruption which could lead to development of drug resistant TB. In any case this view would seem to be supported by Tola, Tol, Shojaeizadeh and Garmaroudi (2015:[5]) whereby they identify being of male gender as a risk factor for non-adherence to TB treatment. However, the findings of the current study seem to suggest that MDR TB affects males slightly less than females.

For physiological factors the researcher had not expected that to increase the odds of males to develop drug resistant TB. Physiological factors seem to be limited to affecting the host's immune response to TB. As Bini, Espinosa, Castillo, Paya´n, Colucci, Cruz, Zatarain, Alfonseca, Pardo, Bottasso and Pando (2014:[5,6]) explain testosterone down regulates immune responses directed against tuberculosis. These include TH1 response, macrophage activation and prostaglandin E2 production. In contrast in aldosterone in females, at the right levels promotes TH1 response, macrophage activation and production of prostaglandin E2.

The male: female ratio is for TB and rifampicin resistant TB is important for policy because it can guide the national TB control program to ensure equitable access to TB services. For instance, if patients of one gender are being notified more than the other, besides reflecting the real epidemiological situation, such indicators may also reflect barriers to

access for the other gender. A TB control program can thus improve performance by identifying the barriers and mitigating them.

5.5.5 Proportions of rifampicin resistance by region

This study's finding show that Lubombo is the region which harbours the lowest percentage of rifampicin resistance while Manzini has the highest. The picture may simply be artefact of sampling whereby in Lubombo there was more missing than any other region. This is a limitation beyond the researcher's control as the study used routinely collected data. Manzini on the other had one site more than the other regions.

The picture also warrants investigation into the real situation of TB in Lubombo region. Given the fact that the population of Swaziland is almost equally distributed among the four region and so is TB burden, the low percentages contributed to drug resistant TB by Lubombo is worrying. It may be indicative of gaps in access to TB services for residents of Lubombo. Further investigation in the form of a survey may be needed.

A survey would be the suitable type of study to determine burden of TB in the region. A survey would allow an understanding of how frequently residents experience symptoms indicative of TB, what kind of healthcare services they seek once they experience such symptoms and the prevalence of TB in the region.

5.6 MECHANISMS OF RIFAMPICIN RESISTANCE

The findings of this study show that dropouts are a more prevalent mechanism for the manifestation of drug resistance than delays. The clinical significance of this is yet unknown. Further the study finds that probe E is the most common location of mutations seen in this study. This true for observations from all Shiselweni F2, Manzini F2 and Manzini F1. The second most popular location of mutations is probe D while the least popular is probe A.

Probe E covers codons 529–533 where the most common mutations are S531L. These findings are like what has been found in other studies. For instance, Kigozi, Kasule, Musisi, Lukoye, Kyobe, Karakazi, Wampande, Joloba and Kateete (2018:[3, 4])

sequenced the RRDR in 45 strains from Uganda. They found that the most frequent loci for mutations were 531, 526 and 516 with 40%, 27% and 9% respectively. The findings of Sanchez-Padilla et al (2015:[1181]) who performed whole genome sequencing on 125 strains from Swaziland, also show that mutation on codon 531 were the most prevalent at 45%. In this study the overall prevalence of mutations in Probe E was 62% which is higher than what Sanchez-Padilla (2015) found. In addition to other reasons discussed above this may be because according to GeneXpert some of the samples included in their study are excluded in this study's analysis because they were detected as rifampicin sensitive.

There are geographic variations in the relative proportions probes in which rifampicin conferring mutations lie. For instance, in contrast to the findings of this study, Chikaonda, Ketseoglou, Nguluwe, Kysiak, Thengolose, Nyakwawa, Rosenberg, Stanley, Mupunga, Hoffman, Papathanasopoulos, Hosseinipour, Scott and Stevens (2017:[4]) find mutations in probe D to be more prevalent than mutations in probe E. This underlines the importance for the pattern of mutations that confer rifampicin resistance to be determined.

The locus of mutations causing rifampicin resistance may have important clinical implications. This is because there is evidence that certain mutations code for low-level resistance so that Mycobacterium TB is resistant to rifampicin but remains sensitive to rifamycins such as rifabutin and rifalazil. Yang, Koga, Ohno, Ogawa, Fukuda, Hirakata, Maesaki, Tomono, Tashiro and Kohno (1998:[623, 624]) identified mutations in codons 514, 516, 518, 526, 529 and 531 as low-level mutations in which strains were resistant to rifampicin but not to rifabutin and rifalazil. In the same study strains with mutations in codons 510, 513, 526 and 531 to be associated with high level resistance to rifampicin rifabutin and rifalazil.

Cavusoglu, Karaca-Derici and Bilgic (2004:[663, 664]) clarify the result of Yang et al (1998) by showing that there are some mutations in the same codons known to be associated with high level resistance may result in low level resistance. Specifically, they showed that in codon 513, the mutations G513P and G513L resulted in low-level resistance even though in general mutations in this codon are associated with high level resistance. Further, for codon 526, the mutations H526T and H526A resulted in low-level resistance. Lastly in codon 531, the mutation S531L resulted in low-level resistance.

For clinical management the nuances in what mutation results in low-level mutations mean that GeneXpert data alone may not be enough to determine when to use rifabutin and rifalazil. There will be need to determine the profile of a country or region before GeneXpert can be used to predict situations in which rifabutin can be used.

In Swaziland in the findings of Sanchez-Padilla (2015:[1181]) almost all the strains (58/59), with mutation in codon 531 had the S531L mutation which is a low-level mutation according to Cavusoglu et al (2004). But all the strains that had mutation in codon 526 had mutations other than the H526T and H526A that re known to be associated with low level resistance. Thus, as already suggested above GeneXpert data may be useful in at least screening if not identifying patients who may benefit from an additional rifamycin in their regimen.

5.7 CONCLUSIONS

GeneXpert data can be used to provide reliable information on proportions of rifampicin resistance in a country. Further it can also be analysed for the proportions of probes in which rifampicin resistance conferring mutations lie. For Swaziland this study found, according to the GeneXpert that the proportions of rifampicin resistance among diagnostic cases for the years 2012–2016 were 10.5%, 10.8%, 11.3, 11,3 and 11,8 respectively. Further this study also found that there was differential risk among gender, places of living and age group. In this regard logistic regression identified that:

- GeneXpert record from females were 1.31 times more likely than those from males to be rifampicin resistant.
- GeneXpert records from persons who lived in Hhohho region were 0.75 times less likely to have rifampicin resistance when compared to those from persons living in Manzini.
- GeneXpert records from persons living in Lubombo were 0.62 and 0.65 times less likely to have rifampicin resistance than those from persons living in Manzini and Shiselweni respectively.
- GeneXpert records from persons aged 0–14 were 0.27 and 0.26 times less likely to have rifampicin resistant TB compared to those from persons aged 15–24 and 25–44 respectively.

- GeneXpert records from persons aged 15–24 were 1.49 more likely than those from persons aged 45–54 to have rifampicin resistant TB.
- GeneXpert records from persons aged 25–44 were 1.56 times more likely to have rifampicin resistant TB than those from persons aged 45–54.

Lastly, the study found that mutations in probe E are the most prevalent mechanism conferring rifampicin resistance in the study sample.

5.8 RECOMMENDATIONS

Based on the findings of this study, the following recommendations are offered. Recommendations describe steps that could be taken to enhance the use of GeneXpert data for surveillance of rifampicin resistance.

5.8.1 Data management

There is a need to minimize the proportion of missing variables to ensure that estimates made from GeneXpert data are as close as possible to the reality. In this study missing data included age, gender, TB treatment history and HIV status. All these variables are important for the national to understand the dynamics of TB and drug resistance in the country. Thus, it is recommended:

To upgrade laboratory examination request forms so that they collect, and capture data collected from presumptive TB patients should at least include the TB treatment history.

To update the LIS to capture at least TB treatment history and if possible, HIV status as well.

In laboratories where paper registers are still being used, there is need to update the registers with fields that can capture the treatment history.

The researcher is aware that in TB treatment registers the treatment history of each patient is also recorded. Thus, the recommendation for the same information to be recorded at the laboratory seems like a duplication of efforts. However, it should be noted that as mentioned before TB treatment registers contain only the bacteriologically

confirmed patients who have started treatment. Some patients detected to have TB at the lab never make it into the registers because of several reasons. Some die before they start treatment. Some emigrate to neighbouring countries. Some choose providers who do not notify the national program which include traditional healers and some private clinics.

There is also a need to extend data management and retention policy to include deep machine data. For GeneXpert it is ideal for the data management policy to harmonize practices of archiving at all labs. During this study it was observed that there were different archiving practices from facility to facility. It is suggested that deep machine be retained for at least ten years and archiving of GeneXpert runs be done cumulatively one time every month. This will result in final yearly archive with data for the runs of all that year. This will simplify the process of data retrieval while ensuring that there is not too long an interval when data is not archived.

5.8.2 Follow-up studies

Taking advantage of the connectivity of GeneXpert instruments through GX-Alert system, it is recommended here to develop an automated system that abstracts information from all GeneXpert machines countrywide to produce a monthly rifampicin resistance surveillance report. This will advance the use of GeneXpert a step closer to routine surveillance of rifampicin resistance.

5.9 CONTRIBUTIONS OF THE STUDY

The main contribution of this study is that it demonstrated that GeneXpert data can be used to provide data on rifampicin resistance for surveillance purposes. Some of the barriers that need to be overcome for the use to be meaningful include updating lab examination request forms and laboratory tools to capture essential variable like TB treatment history of presumptive TB patients.

5.10 LIMITATIONS OF THE STUDY

This study made use of retrospective routinely collected data. As a result, there was limited control on the completeness of data and variables. For instance, the variable TB

treatment history was completely missing. Thus, this study could not demonstrate the effect TB treatment history on development of rifampicin resistance.

92 (1.6%), and 338 (6.2%) of observation had missing values for age and gender respectively. This may affect the conclusions made from the regression model about the differential risk for each of age group and gender. The researcher could have mitigated the impact these missing values by performing multiple imputation, but the procedure was too technical for the researcher. In any case the percentage of missing data for both age and gender was small enough to warrant list wise deletion as the method of choice for dealing with missing values.

The study also basically employs a cross sectional design that covers a long period of time. The limitation of such a design is that risk factors and outcomes are measured at the same time, giving rise to the problem of temporality. Meaning that it becomes unclear what was first, causes or outcomes. This is a problem because one of the criteria for causation is that causes must come before outcomes. However, it should be noted that for relatively fixed causes such as gender this limitation can be considered negligible.

5.11 CONCLUDING REMARKS

Weak or missing surveillance systems for anti-TB drug resistance should be recognized as serious obstacles for global TB control. national programs in countries lacking data on the extent of anti-tuberculosis drug resistance are likely to fail to mount effective TB control programs. The use of rapid molecular diagnostics may provide opportunities for most countries to formulate data driven TB control initiatives. Further national programs may also be able to more persuasively advocate for additional resources where extent of TB and or drug resistance is beyond national means. Lastly, further research is needed that tests models of continuous anti-tuberculosis drug resistance surveillance based on rapid molecular diagnostic or their combinations.

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WHO see World Health Organization.

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ANNEXURES

Annexure A: Ethical clearance from HSREC, UNISA.

UNISA university of south africa

RESEARCH ETHICS COMMITTEE: DEPARTMENT OF HEALTH STUDIES

REC-012714-039 (NHERC)

11 October 2017

HSHDC/718/2017

Tafara Zanamwe
Student 4331-764-2

Dear Tafara Zanamwe

Supervisor: Dr MG Makua
Qualification: D Litt et Phil
Joint Supervisor: -

Decision: Ethics Approval

Name: Tafara Zanamwe

Proposal: GeneXpert data for Rifampicin resistance in Swaziland- A retrospective analysis from 2012 -2016

Qualification: **MPCHS94**

Thank you for the application for research ethics approval from the Research Ethics Committee: Department of Health Studies, for the above mentioned research. Final approval is granted from 11 October 2017 to 11 October 2019.

The application was reviewed in compliance with the Unisa Policy on Research Ethics by the Research Ethics Committee: Department of Health Studies on 6 September 2017.

The proposed research may now commence with the proviso that:

1) *The researcher/s will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.*

2) *Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study, as well as changes in the methodology, should be communicated in writing to the Research Ethics Review Committee, Department of Health Studies. An amended application could be requested if there are substantial changes from the existing proposal, especially if those changes affect any of the study-related risks for the research participants.*

RECEIVED
2017 -10- 24
Executive Dean
College of Human Sciences

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

Open Rubric

3) *The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study.*

4) *[Stipulate any reporting requirements if applicable].*

Note:
The reference numbers [top middle and right corner of this communiqué] should be clearly indicated on all forms of communication (e.g. Webmail, E-mail messages, letters) with the intended research participants, as well as with the Research Ethics Committee: Department of Health Studies.

Kind regards,

Prof JE Maritz
CHAIRPERSON
maritje@unisa.ac.za

Prof MM Moleki
ACADEMIC CHAIRPERSON
molekmm@unisa.ac.za

Prof A Phillipps
DEAN COLLEGE OF HUMAN SCIENCES

University of South Africa
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Telephone +27 12 429 3111 Facsimile +27 12 429 4150
www.unisa.ac.za

Annexure B: Research protocol clearance certificate from NHRRB

Research Protocol clearance certificate

Type of review	Expedited	X		Full Board			
Name of Organization	Student						
Title of study	GeneXpert for rifampicin resistance in Swaziland a retrospective analysis from 2012-2016						
Protocol version	1.0						
Nature of protocol	New	x		Amendment			
List of study sites	[redacted]						
Name of Principal Investigator Names of Co- Investigators	Tafara Zanamwe N/A						
Names of steering committee members in the case of clinical trials	N/A						
Names of Data and Safety Committee members in the case of clinical trials	N/A						
Level of risk (Tick appropriate box)	Minimal			High			
	X						
Clearance status (Tick appropriate box)	Approved	X		Disapproved			
Clearance validity period	Start date	17/01/2018		End date	17/01/2019		
Signature of Chairperson							
Date of signing	17/01/2018						
Secretariat Contact Details	Name of contact officer			Ms Sinsangele Masilela			
	Email address	kalusmasi@gmail.com					
	Telephone no.	(00268) 24043865/24044905					

MINISTRY OF HEALTH
17 JAN 2018
HEALTH RESEARCH REVIEW BOARD
SWAZILAND

Page 1 of 2

Approval Conditions

1	Implementation of approved version of protocol	✓				
2	Reporting of adverse events within 5 days of occurrence	✓				
3	Submission of progress reporting for multi-year studies	Yr 1 N/A	Yr 2 N/A	Yr 3 N/A	Yr 4 N/A	Yr 5 N/A
4	Submission of end of project report (Hard copy)	✓				
5	Submission of end of project report (Soft copy)	✓				
6	Submission of data sets	✓				

List of reviewed documents

Ref.	Documents	Reviewed documents (tick appropriate box)
1	Completed application form	✓
2	Cover letter	✓
3	Evidence of administrative permission to conduct the research by involved institutions/sites (where applicable)	✓
4	Detailed current resume or curriculum vitae of Principal Investigator/s including Principal investigators declaration	✓
5	Summary resume or biography for other investigator(s)	✓
6	Evidence of approval/rejection by other Ethics Committees, including comments and requested alterations to the protocol, where appropriate.	
7	Research protocol (see outline in Annex I)	✓
8	Questionnaires and interview guides (with back-translated versions where applicable)	✓
9	Case report forms (CRFs), abstraction forms and other data collection tools	✓
10	Participant/subjects Information Statement(s) (where applicable)	✓
11	Informed consent form(s) including photographic and electronic media consent statements.	
12	Advertisements relevant to the study (where applicable)	
13	Source of funding and detailed budget breakdown including material and incentives to participants if applicable	
14	Notification form for adverse effects/events.	
15	Point of payment	✓
16	Proof of insurance cover for research subjects in clinical trials or where applicable	
17	Any other special requirements should be stated, if applicable	None

Annexure C: SHLS permission to collect data from laboratories

Telegrams: [redacted]

Telex:
Telephone: (
Fax: (+268 4

20th February 2018

The Laboratory Manager

(See Distribution List attached)

Dear Sir/Madam

RE: Permission to Access and Collect Routine Gene Xpert data for 2012 – 2016)

Tafara Zanamwe, a Master of Public Health Student in the College of Health Sciences at the University of South Africa is herein granted permission to access and collect routine Gene Xpert from your laboratory for Research for the period 1st January 2012 to 31st December 2016 for the above-mentioned purpose. He will need to access the data from: lab registers, Gene Xpert PC and GxAlert.

Please kindly provide him the necessary support and assistance as he conducts this exercise. For any clarification, feel free to contact me.

Yours Sincerely

SWAZILAND HEALTH LABORATORY SERVICE P.O. BOX 6840 MBABANE H100 20 FEB 2018 CHIEF MEDICAL LABORATORY TECHNOLOGIST

Sindisiwe Dlamini

Chief Medical Laboratory Technologist

Swaziland Health Laboratory Service

Annexure D: Request for ethical approval from Swaziland NHRRB

[redacted]

Swaziland

07 November 2017

Dear Sir/Madam

REQUEST FOR PERMISSION TO CONDUCT RESEARCH

I am a registered Master of Public Health student in the College of Health Sciences at the University of South Africa. My supervisor is Dr. MG Makua.

The proposed topic of my research is: *GeneXpert Implementation in Swaziland: Exploration of the GeneXpert data for Rifampicin resistance* The objectives of the study are:

1. To compare Rifampicin resistance patterns by Xpert MTB/Rif with those by phenotypic drug sensitivity testing among patients newly diagnosed with TB for yearly cohorts of 2012 – 2016.
2. To analyze the molecular mechanisms behind Rifampicin resistance by Xpert MTB/Rif probes among patients newly diagnosed with TB for the yearly cohorts of 2012 – 2016.

I am hereby seeking your consent to access and collect routine data for GeneXpert from the following sources:

1. Gx Alert platform for identifying GeneXpert results and the associated cycle threshold data. In the Gx Alert platform all data from GeneXpert instruments is centralized, however demographic data of patients is not available there. For this reason I will also request the next source.
2. Laboratory registers for adding demographic data to the results. Location of Genexpert instrument combined with laboratory number and date of test will be used to match data between Gx Alert data and lab register data.

request the next source.

2. Laboratory registers for adding demographic data to the results. Location of GeneXpert instrument combined with laboratory number and date of test will be used to match data between Gx Alert data and lab register data.
3. My study seeks to determine correlation between Rifampicin resistance status and demographic data. Thus completeness of this data is critical. To enhance completeness of data on age and gender, I propose to make a further search for any missing demographic data in treatment registers

To assist you in reaching a decision, I have attached to this letter:

(a) A copy of an ethical clearance certificate issued by the University of South Africa

Should you require any further information, please do not hesitate to contact me or my supervisor. Our contact details are as follows:

Supervisor Dr. Makua MG |Tel +27 12 429 6524 | Email makuamg@unisa.ac.za
Student Tafara Zanamwe | Tel 2207 9787 | Email 43317642@mylife.unisa.ac.za

Upon completion of the study, I undertake to provide you with a bound copy of the dissertation.

Your permission to conduct this study will be greatly appreciated.

Yours sincerely,

Tafara Zanamwe

Annexure E: Request for permission to collect data from the SHLS chief laboratory technologist

The Chief Laboratory Technologist

Swaziland National Health Lab Services

[redacted]

Nhlangano, S400

07 November 2017

Dear Sir/Madam

REQUEST FOR PERMISSION TO CONDUCT RESEARCH

I am a registered Master of Public Health student in the College of Health Sciences at the University of South Africa. My supervisor is Dr. MG Makua.

The proposed topic of my research is: *GeneXpert data for Rifampicin resistance in Swaziland – A retrospective analysis from 2012 – 2016*. The objectives of the study are:

1. To compare Rifampicin resistance patterns by Xpert MTB/Rif with those by phenotypic drug sensitivity testing among patients newly diagnosed with TB for yearly cohorts of 2012 – 2016.
2. To analyze the molecular mechanisms behind Rifampicin resistance by Xpert MTB/Rif probes among patients newly diagnosed with TB for the yearly cohorts of 2012 – 2016.

I am hereby seeking your consent to access and collect routine data for from laboratories in your network situated at:

a). [redacted]

b). [redacted]

c). [redacted]

d). [redacted]

e). [redacted]

f). [redacted]

g). [redacted]
h). [redacted]
i). [redacted]
j). [redacted]

I am requesting to collect GeneXpert data from the following sources:

1. Gx Alert platform for identifying GeneXpert results and the associated cycle threshold data. In the Gx Alert platform all data from GeneXpert instruments is centralized, however demographic data of patients is not available there. For this reason I will also request the next source.
2. Laboratory registers for adding demographic data to the results. Location of Genexpert instrument combined with laboratory number and date of test will be used to match data between Gx Alert data and lab register data.
3. My study seeks to determine correlation between Rifampicin resistance status and demographic data. Thus completeness of this data is critical. To enhance completeness of data on age and gender, I propose to make a further search for any missing demographic data in treatment registers

To assist you in reaching a decision, I have attached to this letter:

(a) A copy of an ethical clearance certificate issued by the University of South Africa
(b) A copy of ethical clearance issued by the National Health Research Review Board of Swaziland

Should you require any further information, please do not hesitate to contact me or my supervisor. Our contact details are as follows:
Supervisor Dr. Makua MG |Tel +27 12 429 6524 | Email makuamg@unisa.ac.za
Student Tafara Zanamwe | Tel 2207 9787 | Email 43317642@mylife.unisa.ac.za

Upon completion of the study, I undertake to disseminate my findings by providing the National Health Research Review Board a bound copy of the dissertation.

Your permission to conduct this study will be greatly appreciated.

Yours sincerely,

Tafara Zanamwe

Annexure F: 2016 Version of the SHLS general laboratory examination request form

FACILITY NAME:

CODE:

DATE RECEIVED: D D M M Y Y

LAB #

PLACE BARCODE HERE

V2 of 2016

PATIENT DETAILS

DATE REQUESTED | PRIORITY ☐ ROUTINE ☐ URGENT

DATE COLLECTED | TIME COLLECTED

ID NO.

SURNAME

FIRST NAME

DATE OF BIRTH D D M M Y Y AGE:____ SEX: M F

CELLPHONE

WARD

TYPE OF SPECIMEN ☐ Blood ☐ Urine ☐ Stool ☐ CSF ☐ Swab ☐ Other Specify

CLINICIAN'S NAME

FOR PATIENTS UNDER CHRONIC CARE

CURRENT PROGRAM ☐ PRE-ART ☐ ART ☐ PMTCT ☐ RENAL DIALYSIS ☐ DIABETIC ☐ HYPERTENSIVE ☐ OTHER (Specify): _____

PROGRAM #: _____

REASON FOR TEST ☐ DIAGNOSIS ☐ FOLLOW-UP MONTH OF TREATMENT:_____

TUBERCULOSIS

Presumptive TB No.:_____ TB Register No.:_____

On TB Treatment ☐ YES ☐ NO If YES TREATMENT REGIMEN ☐ CAT I ☐ CAT II

TB Specimen ☐ Sputum ☐ Gastric Lavage ☐ Lymph Node Aspirate ☐ Other Specify _____

URGENT

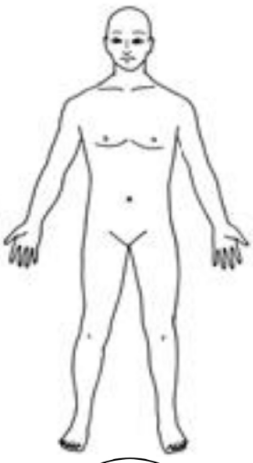
SPECIMEN FOR: ☐ **HISTOPATHOLOGY**

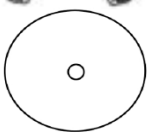
CYTOPATHOLOGY:
☐ **GYNAECOLOGICAL**
☐ **NON - GYNAECOLOGICAL**

Type/Origin of Specimen (specify) .....

RELEVANT CLINICAL INFORMATION (e.g. symptoms, abnormalities, clinical diagnosis)

.....





APPEARANCE OF CERVIX

IS THIS FIRST SPECIMEN? YES/NO .....

PREVIOUS CYTO. REF. NUMBERS .....

PREVIOUS HISTO. REF. NUMBER .....

PREVIOUS SURGERY, CHEMOTHERAPY, RADIOTHERAPY (CIRCLE) ☐ DATE(S).....

FOR FEMALE PATIENTS

L. M. P. ..... **PARITY** ..... **PREGNANT**..... **WKS CONTRACEPTION**..... **POSTMENOPOSAL**.....**YEARS**

PLEASE COMPLETE FOR GYNAECOLOGICAL CYTOPATHOLOGY

SMEAR TAKEN BY:
☐ WOODEN SPATULA ☐ CERVIX BRUSH
☐ CERVIX BROOM ☐ OTHER (Specify) .....

SPECIMENT TAKEN FROM (Circle)
CERVIX ..... **ENDOCERVIX** ..... **VAGINA**..... **VULVA** ..... **ENDOCERVIX**.....

POSTERIOR/LATERAL VAGINAL WALL (FOR HORMONAL ASSESSMENT) .....**VAULT**.....

APPEARANCE OF CERVIX (Circle)
NORMAL ..... **EROSION** ..... **INFLAMED**.....**POLYP** ..... **SUSCIPICIOUS**.....**MALIGNANT**.....

Eswatini Health Laboratory Services. P.O. Box 6840. Mbabane H100 Tel: +268 2404 2190 Fax: +268 2404 8157

Annexure G: List of members of the WHO 2010 expert group

Xpert MTB/RIF Expert Group Members

Dr Richard M. Anthony, PhD
Research Coordinator Tuberculosis
KIT Biomedical Research, Royal Tropical Institute
Meibergdreef 39
1105 AZ Amsterdam
The Netherlands
E-mail:R.Anthony@kit.nl
Area of expertise: Research, Molecular microbiology, Diagnostics development

Dr Rachel Bauquerez
Public Health Officer
Subgroup on Introducing New Approaches and Tools (INAT Subgroup)
The Global Fund
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Area of expertise: Public health, Funding/Donor agency

Dr Catharina Boehme
FIND
Foundation for Innovative New Diagnostics
Avenue de Budé 16
1202 Geneva
Switzerland
E-mail:catharina.boehme@finddiagnostics.org
Area of expertise: TB, Research, Diagnostics development, Developing country experience

Dr Jeremiah Muhwa Chakaya
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Kenya Medical Research Institute
Center for Respiratory Diseases Research
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Kenya
E-mail: chakaya@materkenya.com; chakaya.jm@gmail.com
Area of expertise: TB, Research, Clinical, Public health, Developing country experience, End-user (National TB Control Programme)

Dr Daniela Maria Cirillo, MD PhD
Head, Emerging Bacterial Pathogens Unit
San Raffaele Scientific Institute
Via Olgettina 58
20132 Milan Italy
E-mail: cirillo.daniela@hsr.it
Area of expertise: TB, Research, Clinical, Microbiology, Developing country experience

Dr Frank Cobelens
Senior Epidemiologist
Research Unit KNCV
Parkstraat 17, Postbus 146
2514 CC The Hague
Netherlands
E-mail: cobelensf@kncvtbc.nl
Area of expertise: TB, Research, Epidemiology, Developing country experience

Dr Gerrit Coetzee
Head
National Tuberculosis Reference Laboratory
National Health Laboratory Service
P.O. Box 1038
Cnr Hospital De Karte Street
Braamfontein
2000 - Johannesburg
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E-mail: dcoetzee@phfm.uct.ac.za
Area of expertise: TB, Clinical, Microbiology, Developing country experience

Dr Saidi M. Egwaga
Programme Manager
Ministry of Health and Social Welfare
National TB and Leprosy Programme
P.O. Box 9083
Dar Es Salaam
United Republic of Tanzania
E-mail: tantci@intafrica.com
Area of expertise: End-user (National TB Control Programme), Developing country experience

Ms Christy Hanson
US Agency for International Development (USAID)
USAID/BGH/HIDN/ID
3.7.23, 3rd Floor
Ronald Reagan Building
20523-5900 - Washington, DC
United States of America
E-mail: chanson@usaid.gov
Area of expertise: Public health, Funding/Donor agency, Developing country experience

Dr Doris Hillemann
National Reference Laboratory for Mycobacteria
Research Center Borstel
Parkallee 18
D-23845 Borstel
Germany
e-mail: dhillem@fz-borstel.de
Area of expertise: TB, Research, Clinical, Microbiology, Molecular Microbiology, Developing country experience

Dr Aamir Khan
Director
MDR-TB Control Program
The Indus Hospital
Korangi Crossing
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Pakistan
E-mail: aamir.khan@irdresearch.org
Area of expertise: TB, Clinical, MDR-TB, Developing country experience

Dr Ruth McNerney
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United Kingdom
E-mail: Ruth.Mcnerney@lshtm.ac.uk
Area of expertise: TB, Research, Diagnostics development, Developing country experience

Dr Rick O'Brien
Head of Product Evaluation
Foundation for New Innovative New Diagnostics (FIND)
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Switzerland
E-mail: rick.obrien@finddiagnostics.org
Area of expertise: TB, Research, Diagnostics development, Developing country experience

Dr Giorgio Roscigno
CEO, FIND
Foundation for Innovative New Diagnostics
Avenue de Budé 16
1202 Geneva
Switzerland
E-mail: Giorgio.roscigno@finddiagnostics.org
Area of expertise: TB, Research, Diagnostics development, Developing country experience

Dr Holger Schünemann
Chair, Department of Clinical Epidemiology & Biostatistics
Department of Clinical Epidemiology & Biostatistics
McMaster University Health Sciences Centre,
Room 2C10B
1200 Main Street West
Hamilton, ON L8N 3Z5, Canada
E-mail: schuneh@mcmaster.ca
Area of expertise: GRADE methodologist, Epidemiology

Dr Thomas M. Shinnick
Associate Director for Global Laboratory Activities
Centres for Disease Control and Prevention
1600 Clifton Road
MS-G35, NE
30333 Atlanta,GA
United States of America
E-mail: tms1@cdc.gov
Area of expertise: TB, Microbiology, Molecular microbiology, Research

Mr Javid Syed
TB/HIV Project Director
Community Rep.
Treatment Action Group (TAG)
611 Broadway, Suite 308
NY 10012 - New York, New York
United States of America
E-mail: javid.syed@treatmentactiongroup.org
Area of expertise: Patient/Community representative, Developing country experience

Annexure H: List of members of the WHO 2013 expert group

Sevim Ahmedov (Observer)
Senior Tuberculosis Technical Advisor
United States Agency for International Development
1300 Pennsylvania Ave NW
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Lucy Cheshire
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Gavin Churchyard (Observer)
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Frank Cobelens (Observer)
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Colleen Daniels
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Anne Detjen (systematic reviewer)
Technical Consultant
North America Office
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GENEXPERT DATA FOR RIFAMPICIN RESISTANCE IN SWAZILAND – A RETROSPECTIVE ANALYSIS FROM 2012–2016

by

TAFARA ZANAMWE

submitted in accordance with the requirements

for the degree of

MASTER OF PUBLIC HEALTH

in the subject

HEALTH STUDIES

at the

UNIVERSITY OF SOUTH AFRICA

SUPERVISOR: DR MG MAKUA

JULY 2019

Student number: 43317642

DECLARATION

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